

The effects of carapace coloration and pattern on the predation rate of *Orconectes propinquus* crayfish

By: John M. Lynk

Project Advisor: David M. Lodge

Dept. of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

Abstract

In upper Midwest lake ecosystems, competition between crayfish species often results in niche takeovers which can be devastating in some cases. The three major species involved are *Orconectes virilis*, *Orconectes propinquus*, and *Orconectes rusticus*. Each of these species has a different set of traits than the others. In this experiment, the traits of carapace coloration and pattern were tested to see how they affect the predation of *O. propinquus*. Three treatments of *O. propinquus* crayfish were tested to determine the rate of predation upon them; one treatment was unpainted, one was painted as *O. propinquus* and one was painted as *O. virilis*. Two replicates were performed over protective substrates. One substrate was cobble and the other was macrophyte over sand. Despite several experimental design flaws, the results seem to show that color is a competitive trait in terms of avoiding predation but the color test employed here may not be accurately representative of the intended crayfish species.

Introduction

In many ecosystems, two different species will compete for the same niche. Often, one species will almost entirely displace the less competitive species. Such is the case with crayfish in lakes of the upper Midwest region. Traditionally, lakes of the northwoods were populated exclusively by *Orconectes virilis* and *Orconectes propinquus* crayfish. In the 1970's, a species known as *Orconectes rusticus* infiltrated many of the lakes that *O. virilis* and *O. propinquus* inhabited. The vector by which *O. rusticus* came into place is the live-bait industry used by recreational fishermen (Lodge 2000). While the ecological transition of some lakes from being inhabited mainly by *O. virilis* to being inhabited mainly by *O. propinquus* has been fairly benign, the same cannot be said for the introduction of *O. rusticus*.

O. rusticus exhibits a variety of super-competitive traits that allow it to rapidly expand its population at the expense of *O. virilis*, *O. propinquus*, and other aquatic life. Some of these traits include larger size and greater aggression (Hill and Lodge 1999). These advantages help *O. rusticus* overtake the choicest habitats (rock cobble) and ward off predators better than *O. propinquus* and *O. virilis* (Lodge and Hill 1994). The introduction of *O. rusticus* into a lake is generally thought harmful because *O. rusticus* tends to alter ecosystems by destroying macrophyte cover, reducing habitat heterogeneity, reduce edible invertebrates, and replace *O. virilis* and *O. propinquus* but avoid predation themselves, effectively reducing available food for predators that feed largely on crayfish and other invertebrates (Lodge et al. 2000).

It is important to understand which factors make related species more naturally fit, or competitive, so that vulnerabilities of undesirable species can be used as a management tool. Crayfish exoskeleton coloration and pattern are characteristics that differ from one species to another and may be linked to one species avoiding predation better than another. The inter-species color pattern is important to note as well as color because coloration can vary widely within a given species. For example, given three male *O. propinquus* of the same size, one might be greenish-blue, another orange-rusty, and another tan. Therefore, to adequately give one representation of the visual image of an *O. propinquus* exoskeleton, the location of colors, contrast of light and dark, and shape of markings could matter as much as coloration does.

It is plausible to suggest that exoskeleton coloration and pattern help *O. propinquus* outcompete *O. virilis*. It is the intent of this experiment to prove that if *O. propinquus* crayfish are painted as *O. propinquus* and *O. virilis*, then crayfish predators will preferentially feed on the *O. virilis* color pattern over protective substrates. Also, this experiment incorporates a test for the painting method to

determine whether or not artificial painting can accurately mimic true crayfish carapace coloration and pattern. In this case, the term protective substrates is used to mean substrates where plant matter or rocks provide potential visual and physical barriers to predators. These substrates were chosen because of their mottled appearance, which would likely maximize the crayfishes' natural camouflage. Only *O. propinquus* are used in the experiment in order to isolate inter-species coloration and pattern, as one species might avoid predation better than another on a basis other than coloration and pattern. Finally, because experiments where crayfish have been painted to look like other crayfish are not widely documented, only the visual effect of painting was tested, not potential non-visual effects such as odor or mobility inhibition.

Materials and Methods

Three color pattern treatments were used in this experiment and the test was replicated twice. The color treatments include the control for paint, which was *O. propinquus* unpainted, but with a dab of paint on its tethering apparatus to control for non-visual differences; the control for color, which was *O. propinquus* painted as *O. propinquus*; and the variable for color, which was *O. propinquus* painted as *O. virilis*. Both replicates were put over protective substrates. The two substrates used here were cobble and macrophyte over sand (Kershner and Lodge 1995). The reason that these substrates were chosen is because they have a more mottled appearance than non-protective substrates, which may emphasize the difference in coloration and pattern more than a uniformly colored substrate of sand or mud.

Tethers were used to isolate each crayfish to one area while allowing it some mobility within that area. The tethers used were based on the designs of DiDonato and Lodge (DiDonato and Lodge 1993). Tethers were composed of either 6" x 6" x 1/4" baked ceramic tiles or 4" x 4" x 3/4" corrugated faux-sandstone tiles cross-tied with 1/16" white nylon string. 18" segments of 4 lb test clear monofilament fishing line were attached with clinch knots to the junction of the string on each tile. Size 12 silver-colored crane swivels were tied to the other end of the monofilament line, also using clinch knots, to make the total tether length approximately 14" to 16". (See diagram).

Only male *O. propinquus* were tethered. The average carapace length was 25.6 cm (n = 27). Crayfish were caught by hand, hand net, and wire mesh trap baited with beef liver or yellow perch fillets. All crayfish used were captured within the same drainage, either in Tenderfoot Creek or Tenderfoot Lake of the UNDERC property. Frequently, crayfish were held captive in 5-gallon tanks prior to tethering. Generally, *O. propinquus* proved to be a hardy lab specimen.

Before tethering, crayfish were dried with a cloth towel. To further dry each crayfish, the carapace was wiped with a paper towel soaked in 70% isopropyl alcohol. Once dry, a drop of Instant Krazy Glue (RR) was applied dorsally to the carapace, at the intersection of the head-abdominal and abdominal-abdominal sutures. The barrel and free loop of the crane swivel were pressed down on each glue-laden carapace manually and held in place until dry. Once dry, the tether had to support its captive crayfish's entire body weight for 5 to 10 seconds to be considered secure.

Once tethered, crayfish were painted. Wet'n'Wild Nail Polish (RR) from Wal-Mart was applied manually in appropriate patterns (see color diagram). For *O. propinquus* without paint, the attached crane swivel was painted with color 430B to

control for non-visual effects of paint. *O. propinquus* painted as *O. propinquus* were painted with colors 430B, 441B, and 482B. *O. propinquus* painted as *O. virilis* were painted with colors 439D, 457D, and 422C. Painted crayfish air-dried at room temperature before being placed back into holding tanks.

Complete tethers were transported to each site and hooked by their undersides to 1/16" white nylon lines intended to keep them in place and mark their location. Attached to each line were 10 s-hooks made of heavy-gauge coated copper wire and spaced 36" apart. Each hook was color coded to denote which type of crayfish was tethered to the tile located there (white denoted control for paint, green denoted control for color, and red denoted variable for color). This was done to make replacement possible.

Each line of 10 crayfish was placed in 2-4 ft of water in Tenderfoot Lake. Three lines were used in each replicate, with 10 crayfish of each treatment included. From above with the shore at the top of one's field of vision, crayfish were placed in control for paint, control for color, variable for color order from left to right and top to bottom. Replicates were checked each day for missing crayfish, and these were replaced to prevent under representation of a treatment and subsequent false apparent preference of fish to prey on other treatments. The act of predation was never witnessed at either site, but known crayfish predators that inhabit Tenderfoot Lake include smallmouth bass, rock bass, yellow perch, and sucker. The experiment was run from 14 June to 3 July 2001.

Results

The most important results gained from this study are the survival curves over time for each color treatment of crayfish (See Graph 1 and Graph 2). The most telling patterns are the values of a given survival curve relative to the other curves.

The results for the test of the painting method can be ascertained by comparing the survival curves of the control for paint and the control for color treatments. Parity of values signify that the method of painting is an acceptable way to mimic *O. propinquus* coloration and pattern while disparity signifies that the painting method is an unacceptable way to mimic *O. propinquus* coloration and pattern. On site 1 (macrophyte over sand), the survival curve for the control for paint treatment overlapped the survival curve for the control for color treatment 5 out of 8 days and 8 out of 20 days, and was within one crayfish for 7 out of 8 days and 18 out of 20 days. This similarity between curves suggests that the painting method employed may be suitable to use on a macrophyte over sand substrate. On site 2 (cobble), the survival curve of the control for paint treatment was greater than the survival curve for the control for color treatment. The two curves never overlapped and were only within one crayfish of each other for 3 out of 8 days. Furthermore, the control for paint survival curve overlapped the variable for color survival curve for 7 out of 8 days and was within one crayfish for 8 out of 8 days. This suggests that the painting method was ineffective for *O. propinquus* over a cobble substrate.

Despite the fact that the color and pattern test may be flawed, the results of the test for inter-species competitiveness are nonetheless intriguing. In both replicates, the survival curve for the variable for color treatment was greater than the survival curve for the control for color treatment. At no point did either survival curve overlap. On site 1, the maximum difference between populations was 4 crayfish and

the minimum difference was 1. On site 2, the maximum difference between populations was 3 crayfish and the minimum difference was 1.

Discussion

Several conclusions can be drawn from this experiment. First, carapace color does matter as a competitive trait in crayfish. Secondly, *O. propinquus* painted as *O. virilis* in this experiment is a more competitive color pattern than *O. propinquus* painted as *O. propinquus*. Thirdly, the disparity of the control for paint survival curve and the control for color survival curve on site 2 suggests that the painted representation of *O. propinquus* may not be accurately representative of an unpainted *O. propinquus* crayfish.

The variety of survival curves is indicative of coloration and pattern as competitive traits amongst crayfish species. Especially noteworthy is the consistency of the variable for color survival curve to be greater than the control for color survival curve. If the painted color patterns in this experiment were accurately representative of the intended species, then it could be concluded that *O. propinquus* outcompetes *O. virilis* based on traits other than coloration and pattern. For example, *O. propinquus* might outcompete *O. virilis* based on escape tactics; *O. propinquus* flees predators close to the benthos whereas *O. virilis* suspends itself in the water column when fleeing, leaving itself exposed from all sides and far away from protective substrates (Lodge unpublished observation). However, since the accuracy of each paint job in this experiment is questionable, which natural coloration and pattern is more competitive has not been unquestionably determined here. Nonetheless, the fact that crayfish with one artificially composed color pattern were preyed upon preferentially

in the presence of crayfish with another artificially composed color pattern may be of benefit to the recreational fishing industry, which concerns itself with how to color artificial lures. In this case, it seems that a crayfish lure painted as *O. propinquus* would be superior to one painted as *O. virilis*.

The difference between survival curves of the unpainted treatment and the control for color treatment at site 2 suggests that the painted representation of *O. propinquus* does not accurately reflect natural *O. propinquus* coloration and pattern over all substrates. Whereas the survival curves of the control for paint and control for color treatments were similar over site 1, they were not over site 2. It is possible that vision-dependent predator fish key in on the most contrasting feature of crayfish. Generally, the cobble at site 2 was darker than the sand at site 1. Perhaps the lightest painted color on the control for paint treatment, color 482B, provided a greater contrast than an unpainted carapace would over cobble. In terms of pattern, the painted representation of *O. propinquus* had more well-defined lines than the unpainted treatment, which may have been more noticeable to predators over cobble. Compared to sand, cobble has long, consistent lines of contrast. The short, curvy contrast lines on the control for color treatment of crayfish may have stuck out unnaturally over this substrate. The coloration and pattern-related factors that cause predator fish to target certain crayfish require further study in order to understand why a painted representation of a crayfish matches the natural representation of that crayfish over one substrate but not over another.

The methods employed in this experiment can be improved upon. The lines used to anchor the tiles often became entangled with crayfish tethers, as did aquatic plants. A possible solution to this would be to employ stakes to anchor each tile. Also, the tethers could be shortened to minimize tangling. More basic errors

committed in this experiment were the lack of randomization of the tiles and limited replication. Further research would likely benefit from the avoidance of these errors.

This experiment leaves an array of questions to be answered and opportunities for further experimentation. Primarily, if the experiment was further replicated over each protective substrate then more reliable data could be acquired concerning the issue of whether or not the painted representation of *O. propinquus* adequately represents *O. propinquus* unpainted. Similarly, if more *O. virilis* were available it would be interesting to compare predation rates of painted and unpainted *O. virilis*. Another avenue of experimentation could involve intra-species coloration as a basis for selection. For example, recently molted *O. propinquus* crayfish tend to take on a blue-green coloration which *O. propinquus* predators may take as an indication of that crayfish's recent post-molt stage. Also, egg-bearing female *O. propinquus* take on a mud-brown shade, which may alert fish to their limited mobility caused by clutching the eggs with her tail. This experiment indicates that crayfish carapace coloration and pattern are important factors in crayfish predators' selection of prey, but what aspects of coloration and pattern matter requires further investigation.

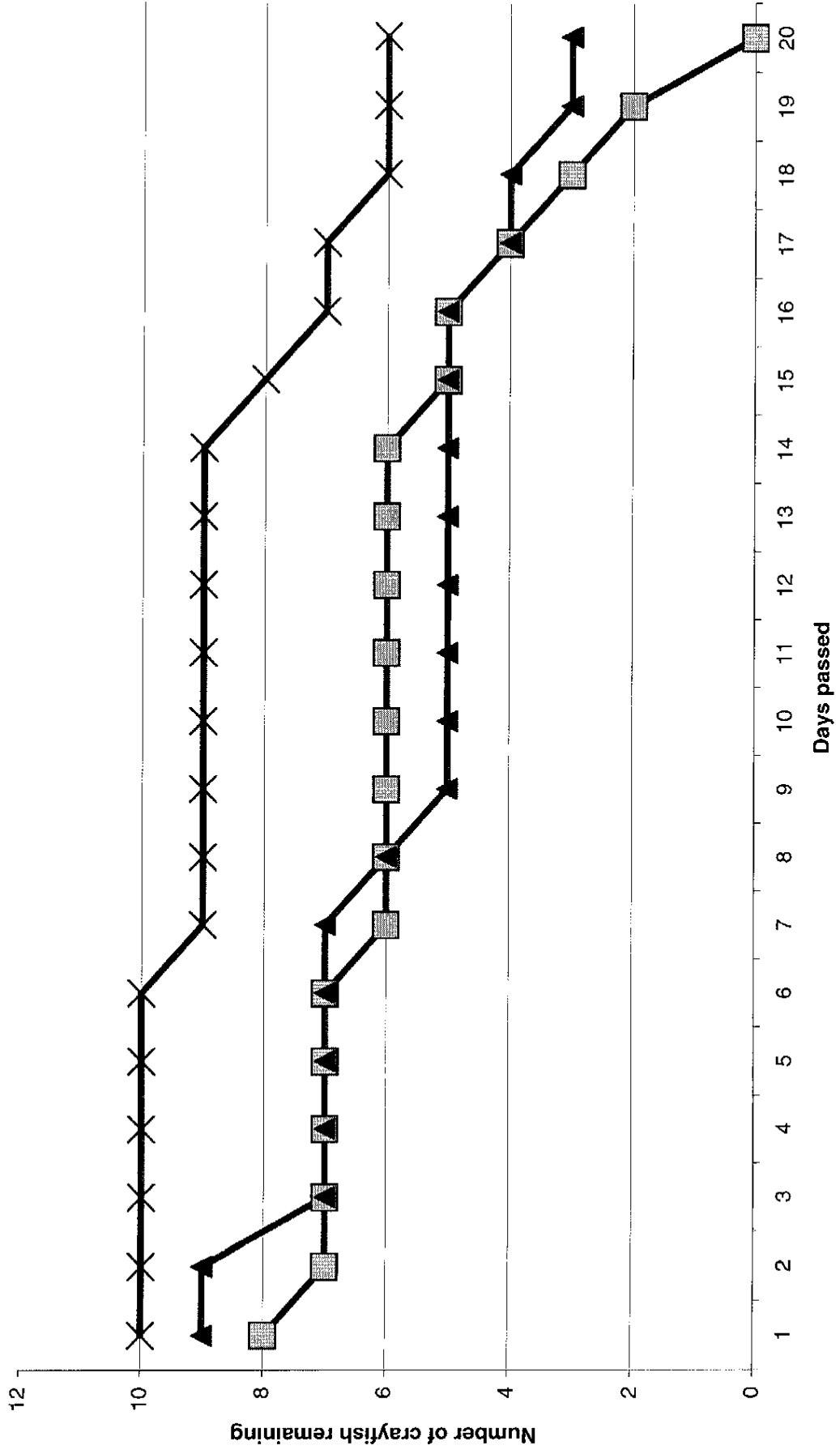
Works Cited

- DiDonato, G.T. and Lodge, D.M. 1993. Species Replacements among *Orconectes* Crayfishes in Wisconsin Lakes: The Role of Predation by Fish. *Can. J. Fish. Aquat. Sci.* **50**: 1484-1488.
- Hill, A.M. and Lodge, D.M. 1999. Replacement of resident crayfishes by an exotic crayfish: The roles of competition and predation. *Ecological Applications* **9**: 678-690.
- Kershner, M.W. and Lodge, D.M. 1995. Effects of littoral habitat and fish predation on the distribution of an exotic crayfish, *Orconectes rusticus*. *Journal of the North American Benthological Society* **14**: 414-422.
- Lodge, D.M. May 2000. Rusties on a Rampage: Rusty crayfish invade Michigan lakes, replace native crayfish species, and rule the lake bottom. *The Michigan Riparian*.
- Lodge, D.M., Taylor, C.A., Holdich D.M, and Skurdal, J. August 2000. Nonindigenous Crayfishes Threaten North American Freshwater Biodiversity: Lessons from Europe. *Fisheries* **25**: 7-20.

Lodge, D.M. and Hill, A.M. 1994. Factors Governing Species Composition,
Population Size, and Productivity of Cool-water Crayfishes. *Nordic J.*
Freshw. Res. **69**: 111-136.

Crayfish survival curve over time (site 1)

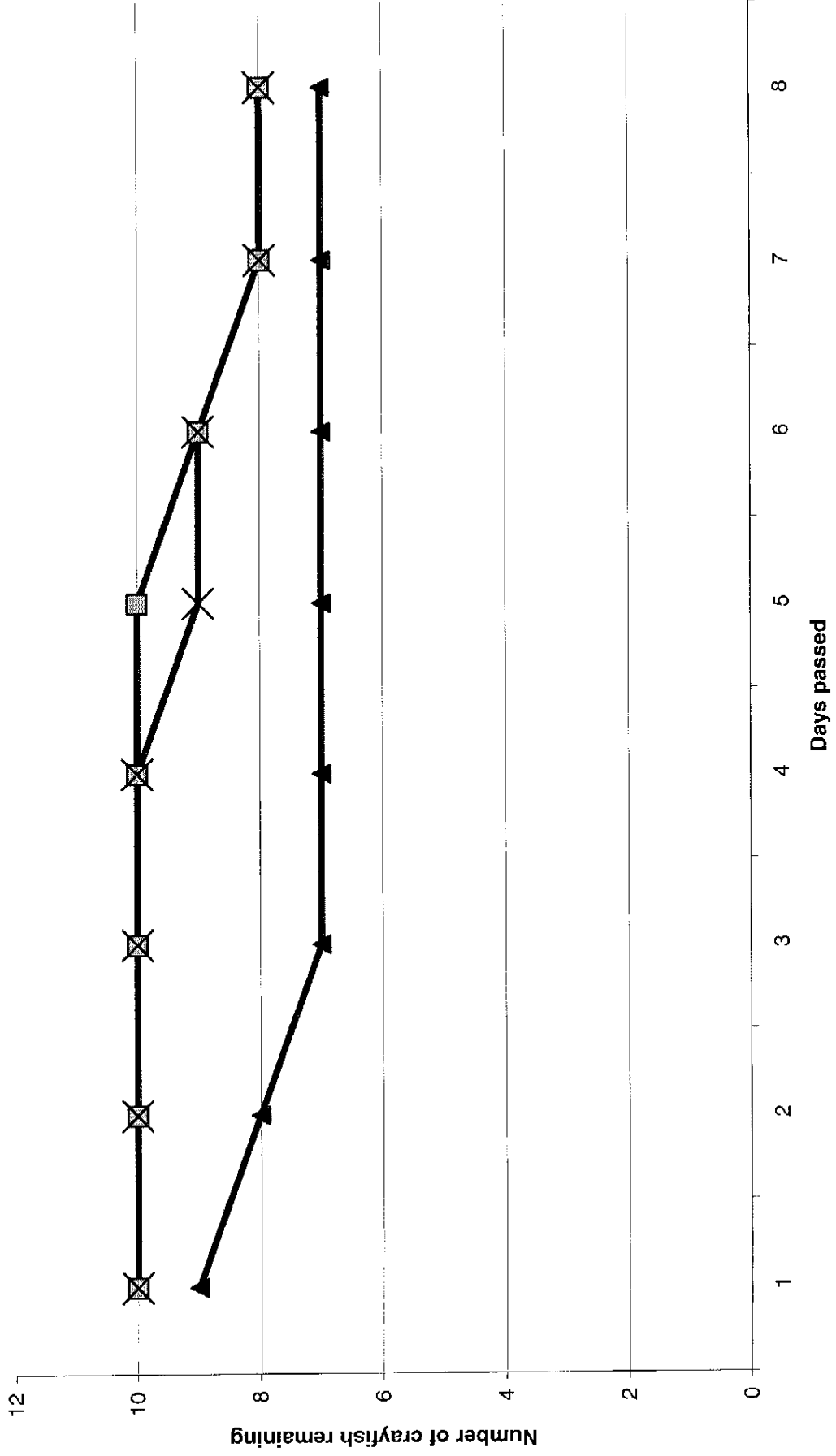
O.p. w/o paint
 O.p. painted as O.p.
 O.p. painted as O.v.



Graph 1. The survival of *O. propinquus* crayfish over a sand/macrophyte substrate was affected by coloration and pattern as is shown here. The similarity of survival curves for *O. propinquus* crayfish painted as themselves and those without paint suggests that the painted representation closely resembles true *O. propinquus* carapace coloration and pattern. The survival curve for *O. propinquus* crayfish painted as *O. virilis* crayfish is greater than the other two survival curves. This suggests that the representation of *O. propinquus* painted as *O. virilis* is competitively advantageous compared to both the natural and painted representations of *O. propinquus*.

Crayfish survival curve over time (site 2)

Legend:
- O.p. w/o paint (square marker)
- O.p. painted as O.p. (cross marker)
- O.p. painted as O.v. (triangle marker)



Graph 2. The survival of *O. propinquus* crayfish over a cobble substrate was affected by coloration and pattern, as is shown here. This graph suggests that the painted representation of *O. propinquus* was much less competitively advantageous than either the unpainted representation of *O. propinquus* or the painted representation of *O. propinquus* as *O. virilis*. The latter two survival curves are strikingly similar. This graph also supports the assertion that the painted representation of *O. virilis* is competitively advantageous compared to the painted representation of *O. propinquus*. It displays a relatively noteworthy disparity between survival curves of the natural and painted representations of *O. propinquus* when this graph is compared to Graph 1. This suggests that the painted representation of *O. propinquus* may not accurately represent *O. propinquus* over cobble.