Color Morphs of the Crayfish *Orconectes virilis*

Robert W. Thacker; Brian A. Hazlett; Laura A. Esman; Craig P. Stafford; Troy Keller

American Midland Naturalist, Vol. 129, No. 1. (Jan., 1993), pp. 182-199.

Stable URL:

<http://links.jstor.org/sici?sici=0003-0031%28199301%29129%3A1%3C182%3ACMOTCO%3E2.0.CO%3B2-W>

American Midland Naturalist is currently published by The University of Notre Dame.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/notredame.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.

Color Morphs of the Crayfish *Orconectes virilis*

ROBERT W. THACKER, BRIAN A. HAZLETT, LAURA A. ESMAN¹,
CRAIG P. STAFFORD AND TROY KELLER

Department of Biology, University of Michigan, Ann Arbor 48109
and

University of Michigan Biological Station, Pellston 49769

ABSTRACT.—Adult *Orconectes virilis* exhibit a variety of colors from yellow to blue to dark brown that are strongly correlated with habitat background color. We used field and laboratory experiments to examine both the proximate mechanisms and the possible functions of this color variation among populations of crayfish in northern Michigan. Field transfer experiments showed that adults can change color, brown morphs becoming blue and vice versa. Light penetration measurements at six field sites indicated relationships between differential wavelength penetration and the colors exhibited by crayfish in a habitat. Holding crayfish in water from different habitats induced color changes while feeding crayfish vegetation characteristic of different habitats did not produce significant color differences. Laboratory predation experiments, field enclosure experiments, and transfer mark-recapture experiments did not indicate any differential survival of morphs that matched or contrasted with the background.

INTRODUCTION

Color morphs of crustaceans have been documented in a number of species. The cobalt-blue morph has been described in lobster (Herrick, 1895) and crayfish species (Newcombe, 1929; Penn, 1951; Smiley and Miller, 1971). Male *Macrobrachium rosenbergii* occur in one of several morphs that vary in color, morphology and reproductive behavior (Ra'anan and Sagi, 1985). Populations of *Daphnia pulex* vary in melanin concentration which serves as protection from ultraviolet radiation (Hebert and Emery, 1990).

In some cases different color morphs appear to be due to genetic differences such as the cobalt-blue morph in several crayfish species (Black, 1974) and the white morph reported for *Orconectes propinquus* (Dunham *et al.*, 1979). Ghidalia (1985) stated that chromatic patterns in crustaceans are genetically determined and that those patterns vary only slightly with environmental changes.

In some instances, different color morphs can be environmentally induced. The hermit crab *Calcinus tibicen* occurs in a reddish-brown and a green morph and individuals have been observed to molt from one morph to the other depending upon diet (Hazlett, 1966). Kent (1901) concluded from field and laboratory observations that crayfish change colors over a period of weeks and appear to match the color of their background.

Possible functions of variation in morphological colors in different individuals are relatively unstudied in crayfish, compared to changes in color or pigment distribution due to chromatophore expansion, *e.g.*, physiological color change (Portmann, 1959; Roberts, 1944 for *Orconectes virilis*). Kent (1901) stated without evidence that the colors of crayfish provide protection from predation. Momot and Gall (1971) noted the 'blue color phase' of *O. virilis* as a relatively rare form in some lakes, mixed with brown-phase individuals. Based upon sampling data, they concluded that there was no difference in survival of the morphs in the lakes containing both forms. Beingesser and Copp (1985) noted color changes with age in

---

¹ Present address: Department of Biology, Albion College, Albion, Michigan 49224.

Procambarus clarkii and differences in field distributions by age that suggested crypsis as a function of the colors.

We have divided this paper into four sections: (A) documentation of the pattern of colors of *Orconectes virilis* in the northern portion of the lower peninsula of Michigan; (B) comparison of behavioral differences between the blue and brown color morphs; (C) experiments examining possible mechanisms leading to the color morphs, and (D) experiments testing possible functions of the colors.

COLOR VARIATION IN DIFFERENT HABITATS

METHODS OF COLOR ANALYSIS

We quantified color using the Methuen Handbook of Color (Kornerup and Wanscher, 1983). The handbook consists of 30 plates, each with a different pure hue in the upper left corner of the plate. Moving horizontally across a plate increases tone while moving vertically down a plate decreases intensity. The bottom row is identical on each plate and is referred to as the grey axis. The 30 plates can be rotated about the grey axis to form a color cylinder (Fig. 1) in which the hues are arranged circularly. Hue, intensity, and tone can be used to describe any observed color and can be represented as a point in three-dimensional space.

We color-coded individual crayfish at two locations on the body: the manus of the right cheliped and the posterior dorsal area of the carapace. We chose these locations because they are relatively large and thus easier to judge the color present and because the colors of the two locations are often quite different from one another. Each individual was therefore associated with a cheliped color and a carapace color. We mapped these colors onto the color cylinder and calculated mean colors for each population or group using a PASCAL computer program written for this study. We calculated the mean cheliped and carapace colors by separately calculating a mean hue, a mean intensity, and a mean tone for each location. Since the hues were arranged in a circle, circular statistics were used to calculate the mean hue.

We compared the colors of two populations or groups by calculating the distance between the mean cheliped color for each population and between the mean carapace color for each population. The difference in color between any two populations was defined as the sum of the absolute values of the differences between each of the components for the two groups. To determine if differences in colors between two groups were statistically significant, we used a basic bootstrap simulation technique (Manly, 1991). Using another PASCAL program, we randomly redistributed the individuals in two populations to form two simulated populations 1000 times. We calculated the distances between the mean cheliped and carapace colors of these populations and generated a two-tailed distribution of possible distances given these random assignments of individuals to two populations. Comparing the actual distance observed to the distribution of simulated distances allowed us to estimate whether the differences in colors between populations were significant.

To avoid damaging the Methuen Handbook of Color while working with aquatic animals, crayfish colors were visually matched to colors found in the Sherwin-Williams Color Answer paint chip sets (1580 different colors). The Sherwin-Williams colors were then visually matched to Methuen Handbook colors by a team of observers. There was a very high degree of consistency among the independent evaluations of color matches.

Adult individuals of *Orconectes virilis* were collected from six locations in northern Michigan: Cecil Bay (on Lake Michigan), Grass Bay (on Lake Huron), Mullet Lake (a moderately large inland lake SE of the University of Michigan Biological Station, UMBS), Burt Lake (a moderately large inland lake S of UMBS), Lake Kathleen and Fontinalis Run. Lake Kathleen is formed by a dam on the Maple River, which runs between Douglas

Methuen Color Cylinder

FIG. 1.—The Methuen color cylinder consists of a grey axis (tone), circularly arranged hues, and an axis indicating increasing intensity of hue

Lake and Burt Lake. Fontinalis Run is a small creek that flows slowly and intermittently into NE Burt Lake.

RESULTS

Each of these six locations has a population of *O. virilis* with characteristic color patterns (Fig. 2). Individual crayfish often had different colors on different parts of the body (Table 1). Crayfish from Cecil Bay and Grass Bay were greenish in color, although some individuals appeared yellow. Individuals from Mullet Lake and Burt Lake were much lighter and were distinctly blue in color. The chelipeds of larger individuals were dark blue. Crayfish from the Maple River in general and from the Lake Kathleen portion of the river in particular were much darker, ranging from medium to dark brown, and the individuals from Fontinalis Run were very dark brown or black. While there is some overlap in the colors shown by individuals from different locations (Fig. 2), individuals could usually be identified as to site of collection by the color patterns shown.

FIG. 2.—Individual *O. virilis* from six locations mapped onto color cylinders. Lake Kathleen (n = 28), Fontinalis Run (n = 4), Mullet Lake (n = 8), Burt Lake (n = 28), Grass Bay (n = 28) and Cecil Bay (n = 11) individuals are illustrated. The length of each line represents tone while the position of the line on the circle is determined by intensity and hue. Individuals identical in their color score overlap on the plots

BEHAVIORAL DIFFERENCES AMONG MORPHS

Studies of some other crustacean color morphs have shown differences in behavior between morphs (Ra'anan and Sagi, 1985). To determine if different color morphs of *Orconectes virilis* differed in behavior, we compared four types of behavior shown by blue-morph *O. virilis* from Burt Lake and brown-morph *O. virilis* from the Maple River. We measured general activity levels during the summer of 1990 at the University of Michigan Biological Station (UMBS), studied substratum color selection and cover selection behaviors in 1990 in Ann

TABLE 1.—Variation in carapace and cheliped colors of *Orconectes virilis* at six locations. Color designations are from the Methuen Handbook

Location	Carapace color	Cheliped color
Lake Kathleen	eye brown	dark brown
Fontinalis Run	black	dark green
Burt Lake	rosewood	campanula blue
Mullet Lake	olive	gray-green
Cecil Bay	rust brown	grey-green
Grass Bay	olive	camel brown

Arbor with animals from the area around UMBS, and examined responses to disturbance pheromone in 1991 at the UMBS.

MATERIALS AND METHODS

Activity levels.—When blue-morph individuals of *Orconectes virilis* from Burt Lake are placed in aquaria in the laboratory, they appear to be more active and more easily disturbed than brown-morph individuals from the Maple River. To test this impression, similar-sized individuals of the two morphs were placed in individual 38-liter aquaria within an hour of capture in the field. The aquaria were visually isolated, well-aerated and contained half a clay pot for a burrow. After 24 and 48 h, the crayfish were observed for 15-min periods during the middle of the day. The posture of the crayfish (body raised, intermediate, or lowered next to the substrata and chelae raised, intermediate, or lowered) and activity (moving about or stationary) were recorded on a portable computer with an event program. These postures have been used in other studies to reflect the level of activation of crayfish (Hazlett, 1985, 1989) and can be important in predator defense by crayfish (Stein and Magnuson, 1976). Using ANOVA, we compared the number of seconds spent in different postures and activities during the observation periods for 10 blue-morph and 10 brown-morph individuals.

Substratum color selection.—Crayfish from Burt Lake (blue morph) and the Maple River (brown morph) were transported to Ann Arbor and placed in holding aquaria. Thirty-eight-liter aquaria were set up each with a substratum of quartz aquarium gravel of uniform size. The gravel in half of each aquarium was black while the other half was white. A linear grid was taped along the outside of each 50-cm-long aquarium with zero at the black end and 50 at the white end. An individual crayfish was placed in the middle of an aquarium during the afternoon, left overnight, and its position in the tank noted three times during the next day (0800, 1200 and 1400 h). The orientations of the aquaria were switched after every four tests to control for possible position effects.

An average position was determined from the three records for each individual and the mean positions compared for the brown and blue morphs with a t-test. Thirteen individuals of each morph were tested. The mean size of animals tested was 40.3 mm carapace length for the Burt Lake specimens and 35.7 mm for the Maple River specimens.

Cover selection.—To compare the responses of individuals of the two morphs to transmitted light, crayfish were placed in an aquarium with plastic "burrows." These "burrows" were hemicylinders of clear plastic 126 mm long and 62 mm in diam. Half of the length of the hemicylinder was darkened with a black felt-tip pen and the other half left clear. Four crayfish (two brown morphs, two blue morphs) were tested at a time in an aquarium with four "burrows" on a substratum of sand and mixed gravel. The aquarium was adjacent to a window and the tests were conducted in November 1990. The position of each crayfish (carapace under the blackened half of the burrow, carapace under the clear half, crayfish not in the plastic burrows) was recorded at 2-h intervals four times during 1 day following placement in the aquarium.

Disturbance pheromones.—When crayfish are disturbed (for example, by a predator or by aggression), they emit chemicals that individuals of *Orconectes virilis* can detect (Hazlett, 1985). Earlier experiments demonstrating this behavioral response used brown morph *O. virilis* from the Maple River. Individuals of other species (*O. propinquus*, *O. rusticus*) do not respond to the chemicals although *O. rusticus* readily emits the chemicals (Hazlett, 1989, 1990). We investigated whether the blue morph of *O. virilis* also responded to disturbance pheromones.

The same methodology employed in earlier tests was used with blue-morph *Orconectes*

TABLE 2.—Average (SD) number of seconds spent by *Orconectes virilis* from Burt Lake and the Maple River ($n = 10$ for each location) in different postures during 15-min observation periods 1 and 2 days after being placed in the laboratory

Color morph	Moving	Posture		
		Lowered	Intermediate	Raised
Brown morph	49 (61)	728 (257)	84 (144)	0.4 (1.7)
Blue morph	159 (186)	174 (319)	486 (346)	139 (266)

virilis from Burt Lake (Hazlett, 1985, 1989). Individual crayfish were placed in 38-liter aquaria in well-aerated water with half of a clay pot for a burrow. Aquaria were visually isolated from each other. After 3–5 days in the laboratory individuals were tested under three conditions: introduction of self water, from the same aquarium containing the test crayfish (Hazlett, 1985), introduction of water from an aquarium containing undisturbed blue-morph *O. virilis*, and introduction of water from an aquarium containing blue-morph *O. virilis* disturbed just prior to a test by being chased around the aquarium by the investigator. Water was introduced at the rate of 20 ml/min with a peristaltic pump for 8 min during which time the postures of the test *O. virilis* (raised, intermediate, lowered) were recorded on a computer equipped with an event recorder program. The number of seconds spent by the crayfish in the different postures under the three conditions was compared by ANOVA. Twenty replicates were run with only one test per day for any individual.

RESULTS OF BEHAVIORAL DIFFERENCES IN COLOR MORPHS

Activity levels.—The activity of the two morphs of *Orconectes virilis* in the laboratory differed significantly (Table 2). There were no significant differences between the 24- and 48-h observation periods in either seconds spent moving or in particular postures ($P > 0.05$ by both the interaction term of session $\times$ source from ANOVA and by t-test); therefore the first and second period data were combined. The blue-morph *O. virilis* spent more time moving ($t = 2.5$, $P = 0.017$), less time in the lowered postures ($t = 6.0$, $P < 0.0001$), and more time in the raised postures ($t = 2.3$, $P = 0.025$).

Background selection.—The average position of crayfish along a black-white gradient of background color differed significantly for the two color morphs. Both morphs were recorded more frequently on the darker end of the tank; however, Maple River *Orconectes virilis* were found in the black substratum portion of the aquaria significantly more frequently than *O. virilis* from Burt Lake (mean position was 2.8 cm for Maple River, 18.7 cm for Burt Lake, $t = 2.9$, $P = 0.008$).

Cover selection.—The distribution of individuals of the two morphs relative to levels of transmitted light did not differ. Counting the four location records for each of the 12 individuals of each morph, the Maple River individuals were under the darkened half of the plastic burrows 20 times, under the clear half once, and were not in a burrow 27 times. The Burt Lake animals were found 20 times in the dark half and 28 times not in a burrow. Obviously there was no significant difference in location of the two morphs in this laboratory situation.

Disturbance pheromone.—Blue-morph individuals of *Orconectes virilis* responded to disturbance pheromones from conspecifics by an increased rate of movement, more time spent in the intermediate postures and less time spent in the lowered postures (Table 3). There were no significant differences between the time spent in activities in the self water and undisturbed conspecific conditions (all t-test values associated with $P > 0.05$). There were

TABLE 3.—Average (SD) number of seconds spent in different activities during 8-min observation periods by 20 individuals of the blue morph of *O. virilis* when one of three sources of water was introduced to a home aquarium

Source of water	Moving	Posture		
		Lowered	Intermediate	Raised
Self water	45 (83)	254 (207)	186 (182)	32 (94)
Undisturbed conspecifics	26 (44)	305 (109)	141 (165)	30 (75)
Disturbed conspecifics	77 (77)	194 (169)	234 (157)	43 (82)

significant differences between the undisturbed and disturbed conspecifics condition in movement ($t = 2.1$, $P = 0.043$) and time spent in lowered postures ($t = 4.3$, $P = 0.001$). The difference in the time spent in the intermediate position was not significant in this comparison ($t = 0.09$) while there was a difference comparing the responses to self water and water from disturbed conspecifics ($t = 3.3$, $P = 0.004$). Since animals are categorized as being in one of the three classes of postures, only two of the three may be compared statistically.

MECHANISMS LEADING TO THE COLOR MORPHS

The general mechanisms that could be involved in determining the colors of crayfish are genetic differences between populations, environmental induction of the observed colors, and an interaction between genetic differences and environmental factors. The following experiments and measurements were conducted to test if color differences could be a result of environmental induction. The four tests involved: (a) field transfer experiments; (b) rearing in water from different habitats; (c) rearing on different diets, and (d) correlation of crayfish colors with patterns of light penetration in nature. These experiments focused on individuals from just two locations, Lake Kathleen and Burt Lake. These two lakes differ in several limnological parameters (Gannon and Paddock, 1974), including vegetation, water chemistry and light penetration. Differences in aquatic vegetation allow for possible differences in diets for crayfish in each location.

METHODS

Field transfers.—A field transfer experiment was conducted during the summer of 1991 to see if animals placed in new environments could change colors. Cross-transfers of individuals of *Orconectes virilis* were made between Burt Lake and the Lake Kathleen portion of the Maple River. Crayfish were placed in enclosures made from ½" mesh hardware cloth (2' × 3' × 8" high). Enclosures were secured in the field with a stake, and 15 medium-small *O. virilis* (15–20 mm carapace length) were placed in each of three replicates at both locations. Enclosures placed in Burt Lake had 10 individuals from the Maple River and five from Burt Lake, while enclosures placed in the Maple River had 10 individuals from Burt Lake and five from the Maple River. All were scored for carapace and cheliped color as described above, and one uropod was clipped to identify source location.

We placed enclosures in three locations in the N end of Burt Lake chosen to minimize possible disturbance by/to humans. Two were located in the NE portion of the lake ca. 5 m apart and the third was almost a kilometer away in the NW portion of the lake. In Lake Kathleen, two replicates were ca. 10 m apart in the western part of that impoundment and the third about 500 m to the E. In all cases the enclosures were in ca. 50 cm of water. Food was added to the enclosures periodically in the form of the surrounding macrophytes (Maple River) or algae-encrusted rocks (Burt Lake) from the immediate environment. Thus the

crayfish were exposed to the local environmental conditions in the two locations as much as possible. The crayfish were left in the enclosures between 3 (Burt Lake) and 4 (Maple River) wk and the crayfish were then counted and scored for color. We compared the mean colors of groups before and after exposure to field conditions. The null hypothesis was that there would be no difference in the mean colors of crayfish before and after treatment.

Effects of water.—Given the results of the field transfer experiments (*see below*), we wanted to determine which aspects of the field situation might contribute to the changes in color. Water chemistry could influence color in crayfish by interacting with the exoskeleton during the hardening process after a molt. Crayfish exoskeletons are permeable to water, especially after molting, when crayfish are soft for several days (Stevenson, 1985). The exoskeleton may interact with calcium ions, tannins, or other substances in lake or river water. To determine if color change could result from a difference in water chemistry, we held crayfish in the laboratory in water from Burt Lake and Lake Kathleen.

We collected 54 young-of-the-year *Orconectes virilis* from Lake Kathleen and held 27 in water from Lake Kathleen and 27 in water from Burt Lake for 2 wk. They were held in 8 liters of water in 38-liter aquaria (eight aquaria for the two treatments) and exposed to overhead lighting in the laboratory. The crayfish were fed commercial shrimp pellets and were checked daily for molting. Individuals were marked by removing one of the walking legs which allowed identification of the molts and accelerated the molting process. Overall, 50% of the individuals molted during the 2-wk period. The crayfish were color-coded at the beginning and the end of the experiment. The mean colors of groups were compared before and after being held in different water. The null hypothesis was that there would be no difference in the mean colors of crayfish before and after treatment and that there would be no difference in the effects of the water from different sources.

Effects of diet.—Dietary differences could be responsible for the observed differences in crayfish color because the major pigment in the exoskeleton of crayfish, astaxanthin, is synthesized from carotenoids in food items (Ghidalia, 1985; Stevenson, 1985). Astaxanthin alone produces a red color, but in association with proteins it may produce other colors including blue and green (Ringelberg, 1980). If crayfish consume different amounts or types of carotenoids, they may differ in the amount of astaxanthin incorporated into the exoskeleton, resulting in color differences.

A common diet item in Lake Kathleen is *Elodea canadensis*, which is scarce in Burt Lake, while *Najas flexilis* is commonly eaten by crayfish in Burt Lake, but is not common in Lake Kathleen (pers. observ.). We assayed the carotenoid content of these plants by extracting a known wet weight of plant tissue in 90% acetone with a tissue homogenizer. After centrifuging the extract, the supernatant was analyzed on a Bausch & Lomb Spectronic 710 spectrophotometer at 480 and 750 nm, following the procedure of Wetzel and Likens (1991) to estimate the amount of total carotenoids. This procedure was replicated four times for each species. *Elodea canadensis* contained much higher levels of carotenoids (0.451 ± 0.102 micro Specific Pigment Units per milligram wet weight of plant tissue) than *Najas flexilis* (0.137 ± 0.035 units).

We held individuals of *Orconectes virilis* on a diet of either *Elodea canadensis* or *Najas flexilis* for 2 wk. Twenty-eight adult crayfish (14 males, 14 females) were collected from Lake Kathleen and 28 from Burt Lake. Seven males and seven females from each lake were held on each diet in 38-liter aquaria filled with 8 liters of well-water from the UMBS. Eight aquaria were used with seven crayfish in each. The colors of each individual's carapace and chelipeds were recorded at the beginning and end of the 2-wk holding period. After recording the final colors, a 1-sq-cm portion of the carapace and cheliped exoskeleton was removed from each individual and homogenized in 100% methanol to extract pigments. The

FIG. 3.—Color of individual *O. virilis* before and after being held in water from Lake Kathleen (filled squares) or Burt Lake (open circles)

absorbance of these extracts was measured at 474 nm on a Bausch & Lomb Spectronic 710 spectrophotometer to assay for astaxanthin content using the procedure described by Hairston (1979).

Variation in light transmission.—If crayfish match the background color of their environment, the quality and quantity of incident light and/or light reflected from the background could indicate which color to match. We measured light attenuation of total wavelengths as well as the red, green and blue wavelengths at six locations (see Fig. 2). We used a LICOR LI-185B light meter to take readings at three depths at three sites in each body of water for each category of wavelengths. We calculated the extinction coefficient (η) of each lake for each group of wavelengths using the simple linear regression

$$\ln I_z = -\eta z$$

where I represents intensity and z represents depth (Wetzel and Likens, 1991).

RESULTS

Field transfers.—Crayfish cross-transferred into enclosures for 3–4 wk were capable of changing colors. While some individuals did not appear to be different from when they were placed in the enclosures, others (about half) definitely changed. Some of the animals from Burt Lake placed in the Maple River became browner (mean color of those animals changed significantly, $P < 0.01$ comparing the magnitude of color change from before to after treatment) while some of the animals from the Maple River placed in Burt Lake became bluish or purple (mean color changed significantly, $P < 0.01$).

Effects of water.—If water chemistry influences the color of crayfish, we expected the crayfish held in Lake Kathleen water to either not change or become browner and the crayfish held in Burt Lake water to become lighter and more blue. Individuals from both sources became lighter ($P < 0.01$) which could have been caused by a variety of factors, including the diet the animals were fed. However, we found that the individuals held in Lake Kathleen water became even lighter than the individuals held in Burt Lake water ($P = 0.04$) (Fig. 3). The only difference in treatment of the two groups was the source of the water in which they were held.

Effects of diet.—In the diet experiments, we expected that crayfish fed *Elodea canadensis*

TABLE 4.—Analysis of variation in astaxanthin content of the (A) cheliped and (B) carapace of crayfish

Source	ss	df	ms	F	P
(A) Cheliped					
Location	0.017	1	0.017	96.864	0.000
Diet	0.001	1	0.001	5.409	0.026
Location × diet	0.001	1	0.001	5.422	0.025
Error	0.006	37	0.000		
(B) Carapace					
Location	0.048	1	0.048	61.283	0.000
Diet	0.004	1	0.004	5.223	0.028
Location × diet	0.003	1	0.003	4.136	0.049
Error	0.029	37	0.001		

would become darker and redder than crayfish fed *Najas flexilis* and have a higher content of astaxanthin. No differences were found between the sexes, so the data were pooled by sex. There was no difference in color between crayfish from Lake Kathleen which were fed the two diets ($P > 0.35$) at the beginning of the experiment nor at the end of the experiment ($P > 0.35$). The crayfish from Burt Lake differed in color between the groups fed the two diets at the beginning of the experiment ($P < 0.05$) but this difference was not present between diet groups at the end of the experiment ($P = 0.98$). However, for both cheliped and carapace exoskeleton, more astaxanthin was present in Lake Kathleen individuals than in Burt Lake individuals. Among Lake Kathleen individuals, more astaxanthin was present in the exoskeletons of individuals fed *E. canadensis* than individuals fed *N. flexilis* (Table 4; Fig. 4).

Variation in light transmission.—Total light attenuation was significantly different between

FIG. 4.—Amount of astaxanthin present in the carapace of individuals of *O. virilis* after being fed either *E. canadensis* (open bars) or *N. flexilis* (hatched bars). Amounts expressed as optical density per milligram wet weight of exoskeleton

FIG. 5.—Total light penetration in six locations in northern Michigan represented by the negative value of the extinction coefficient

locations (ANOVA, $F = 7.907$, $P < 0.001$) (Fig. 5). In addition, three patterns of light attenuation were found for the red, green and blue wavelengths (Fig. 6). Lake Kathleen and Fontinalis Run transmitted red light best; Mullet Lake and Burt Lake transmitted green light best, and Cecil Bay and Grass Bay transmitted blue light best. Comparing these patterns of wavelength transmission to the colors of the crayfish in these locations, the lighter-colored crayfish were found in lakes in which blue or green light transmitted maximally, while the darker colored crayfish were found in lakes in which red light transmitted maximally.

EXPERIMENTS ON POSSIBLE FUNCTIONS OF COLORS

MATERIALS AND METHODS

Survival in enclosures.—Blue- and brown-morph individuals were held in enclosures in Burt Lake and the Maple River for 3–4 wk. The number of individuals of each color morph was counted at the end of the period.

Mark-recapture transfers.—During the summers of 1990 and 1991, experiments were done to test the hypothesis that crayfish color would influence recapture rate. A basic assumption is that recapture rate would reflect relative rates of survivorship in a given environment. Individuals of *Orconectes virilis* were collected at night in either Burt Lake or the Maple River. We sexed, measured carapace length, and individually marked each with a pattern of pleural clips (Hazlett *et al.*, 1974). In 1991 each crayfish was also coded as to

FIG. 6.—Extinction coefficients for red, green and blue wavelengths of light at six locations in northern Michigan

carapace and cheliped color. The crayfish were then either returned to the area of collection or transferred to the other location. In 1990 all four possible combinations were established: Burt Lake animals returned to Burt Lake, Burt Lake animals released in the Lake Kathleen portion of the Maple River, Maple River animals returned to Lake Kathleen, and Maple River animals released in Burt Lake. In 1991 Lake Kathleen was the only release/recapture site.

Subsequent surveys of Burt Lake and Lake Kathleen recorded recaptures of individuals of known sex, size and original location. Some individuals were captured in Lake Kathleen via baited traps and a few by tactile search of burrows along the shore of Lake Kathleen, but the primary means of recapture was by visual detection of animals at night aided by headlamps.

The recapture data were analyzed as to the relative frequency of recapture of the two color morphs as well as the relationship of size to recapture probability. Data were analyzed both by the counts of individuals who were recaptured at least once during the summer and by calculation of the number of days of survival for recaptured individuals (date of last recapture minus date of initial marking) according to color and size. Initial markings were done on 2 July in 1990 and 26 June in 1991 and surveys continued to 19 August in both years.

Predation.—To examine the relative rates of predation on blue- and brown-morph *Orconectes virilis*, a trough (17 × 55 cm wide × 4 m long) made of opaque plastic was set up in the laboratory at the UMBS with a continual supply of running lake water (from Douglas Lake) and substrata characteristic of either Burt Lake or the Maple River. Sand and pebbles from the two areas were collected and placed in 100-cm sections of the trough creating relatively light-bottomed or dark-bottomed backgrounds. Each section contained a potential crayfish predator, either a largemouth or smallmouth bass. The fish were caught from

TABLE 5.—Results of mark/recapture experiments, number of individuals released and recaptured at least once. Known days of survivorship calculated as date of last capture minus date of initial release. Numbers in parentheses indicate the number of cases expected if there were no differences between color morphs and taking into account the different number of morphs released in each environment (for recaptures) and when they were released (for expected days of survival)

	Number released	Size mm C.L.	Number recaptured	Sum days survived
1990 Data				
Burt-Burt	122	45.1	6 (3.9)	78 (50)
Maple-Burt	66	37.5	0 (2.1)	0 (28)
Maple-Maple	142	33.3	9 (20.4)	81 (232)
Burt-Maple	102	43.9	26 (14.6)	310 (159)
1991 Data				
Maple-Maple	107	36.4	9 (15)	170 (272)
Burt-Maple	107	42.1	21 (15)	342 (240)

Note added in proof: Mark-recapture of blue and brown morphs of *O. virilis* was conducted during the summer of 1992 at a different site along the Maple River. This site about 1 km upstream from Lake Kathleen is a fast-flowing, riverine habitat rather than the lake environment of the empoundment at Lake Kathleen. One hundred fifty-one individuals were marked and released (28.5% blue morphs) and 45 individuals were recaptured over the summer. Of these, 15 individuals were blue morphs which is obviously not different from the number expected (12.8) if there were no differential recapture by color. Thus, the results obtained previously from a lake-like portion of the Maple River were also obtained in a habitat with a different array of potential sources of mortality for crayfish.

Douglas Lake and allowed to acclimate to the laboratory situation (and increase hunger level) for at least 4 days prior to testing.

Each test consisted of the simultaneous introduction, on two sides of the trough, of a brown-morph and a blue-morph *Orconectes virilis*. The crayfish were allowed to acclimate for 1 min and then a partition separating the fish from the rest of the chamber was raised. The crayfish were matched in size to within 1 mm carapace length (c.l.) and were small enough (mean size = 31.2 mm c.l.) that the fish could easily eat them. Observers watched from both sides and the time to first predatory strike and which morph was attacked first was recorded. If no strike was observed in 30 min, the test was abandoned. Success of predation (crayfish eaten or not eaten) was also recorded. All tests were run in the late afternoon.

RESULTS

Survival in enclosures.—There was no difference between the two color morphs in survivorship in the enclosures placed in the two environments, although there was a very clear difference in survivorship between the two environments. In Burt Lake only four out of 15 (26%) blue morphs survived while 12 of the 30 (40%) brown morphs survived. In the Maple River, 27 of 30 (90%) of the blue morphs survived and 12 of 15 (80%) brown morphs. Clearly, matching or not matching the background color of the environment had no effect upon survival in medium-small *Orconectes virilis* in these habitats over a period of a month.

Mark-recapture transfers.—A total of 432 animals were marked during the summer of 1990. Forty-one individuals were recaptured at least once during 1990 and one marked in 1990 was recaptured in 1991. There were clear differences in the probability of recapture both between habitats and between color morphs (Table 5). The number of recaptures in

Burt Lake was very low and little can be concluded from these numbers regarding differential recapture or survival of color morphs. Recaptures were higher in Lake Kathleen and the blue-morph individuals were clearly recaptured at a higher rate (chi-square comparing observed and expected number of individuals recaptured = 15.2, $P < 0.01$). However, this result is complicated by the difference in average size of the animals from the two habitats. Burt Lake *Orconectes virilis* released in Lake Kathleen were larger than those captured and replaced in the Maple River and there is a significant relationship in the 1990 data between crayfish size and days of known survival (regression $F = 93$, $P < 0.001$).

In 1991 we focused on Lake Kathleen (given the low recapture rate overall in Burt Lake) and attempted to equalize the size of the crayfish from the two habitats. While the mean sizes were not equal, they were closer and there was no correlation between crayfish size and days of known survival ($F = 0.212$, $P = 0.649$). There were still more individual blue morphs recaptured than brown morphs (chi-square = 4.8, $P < 0.05$). There was no difference in the average number of days of known survival for the two morphs. Blue morphs that were recaptured averaged 16.3 days between first and last capture and brown morphs averaged 18.9 days. The overall result from both years of mark-recapture experiments was that matching background did not result in longer survival in the field in the Maple River.

Predation.—The laboratory experiments on fish predation yielded 16 trials which involved a clear strike by the predator directed at one of the crayfish. In eight cases the fish ate the crayfish. Smallmouth bass were the predators in 11 cases, largemouth bass in five cases. Nine trials were on Burt Lake background and seven on Maple River background. There was no difference in the average size of the blue (mean = 31.3 mm c.l.) and brown (mean = 30.9 mm) morphs ($t = 0.48$, $P > 0.10$). In eight cases the brown crayfish was attacked first and in eight cases the blue crayfish was attacked first. In eight cases the crayfish attacked first contrasted with the background (brown morph on Burt Lake background or vice versa) and in eight cases the fish attacked the morph matching the background. And in eight cases the attacked crayfish was the larger of the two and in eight cases it was the smaller.

The only parameter that hinted at selectivity by the predators was the time to first strike. When the crayfish that matched the background was struck first, the time between introduction and first strike was 5.0 min while it was 3.5 min if the struck crayfish contrasted with the background. However, this difference was not significant, $t = 0.7$, $P > 0.10$). Similarly, when the smaller of the two individuals was struck first, the time to first strike was 5.6 min while it was 2.9 min when the larger was struck first ($t = 1.4$, $P > 0.10$).

DISCUSSION

Color variation.—The colors of individuals of the crayfish *Orconectes virilis* are highly variable. Clearly this variability is not random since much of the variance in color is explained by consideration of the environments in which the animals are found (Fig. 2). The significant differences between *O. virilis* in the Maple River and Burt Lake are all the more striking when one considers the direct connection of these bodies of water. Despite searching at the mouth of the Maple River where it empties into Burt Lake, no brown-colored *O. virilis* have been found in Burt Lake. While part of the link between location and color could be due to genetic differences (this possibility was not tested in this study), environmental induction of the colors shown by individuals clearly does occur. The transfer experiments demonstrate that changes in color of adult crayfish can occur over a period of weeks. Some of the larger individuals transferred to Lake Kathleen from Burt Lake in the mark-recapture experiments also changed colors over the course of the summer. The ability to change colors had been suggested by the observations of Kent (1901) and Beingesser and Copp (1985).

Mechanisms of induction.—Our attempts to determine which aspects of the environment

are critical for color induction were partially successful. In these experiments (diet and water chemistry), the relatively short time of treatment obviously limited the strength of our results. This is especially true given the restriction that these color changes occur during molting. Since many individuals did not molt during the experimental period, the small (or insignificant) differences must be interpreted with caution. Our method of analyzing color differences was particularly conservative since we included all individuals in any particular group in the estimate of mean color, not just those that had molted. We did not include only molted individuals in the analyses because we could not be certain that an individual had molted and eaten its shed exoskeleton. In spite of the conservative nature of our estimates and the limited time period, significant induction effects were observed.

The higher levels of astaxanthin in the exoskeleton of crayfish fed *Elodea canadensis* compared to those fed *Najas flexilis* could translate to observable color differences if more individuals had molted or the crayfish had been fed for a longer time. The fact that water chemistry affected colors over such a short period of time points to potentially strong effects over several molts. The fact that 3–4 wk of exposure to all the conditions (diet, water and light) of a different environment resulted in such clear color changes by some crayfish suggests that several factors may be involved in the induction process.

One of the most striking potential mechanisms underlying color induction may be light. The relationship between the pattern of wavelength attenuation in different locations and the colors of crayfish found in those locations is very suggestive. Crayfish have both blue-sensitive and red-sensitive photoreceptors (Wald, 1968), making direct induction effects by differential activation of photoreceptors a possible mechanism. However, differences in diet (light environment affecting macrophyte or algal species present) or water chemistry cannot be ruled out. Features of water chemistry (suspended particulates, etc.) determine the patterns of wavelength attenuation, thus the relationship between attenuation patterns and colors of crayfish could be just a correlation. Further experimental separation of these factors is clearly needed.

Although environmental factors can influence color change, genetic differences between populations may also be responsible for variation in color. Breeding experiments combined with rearing under different conditions are needed to determine the extent and nature of possible genetic influences.

Functions of colors.—The results of experiments designed to test for functions of the colors observed in different environments did not yield clear positive answers. The results and interpretations of each of our experiments can be questioned. While bass are known to consume crayfish (Momot and Gowing, 1977; Stein and Magnuson, 1976), other potential predators may be important and our laboratory situation may not translate readily to the field. Documentation of differential predation of crayfish via tethering experiments have been useful in other systems (Mather and Stein, *in press*). The lack of differential survival in the enclosure experiments could be attributed to the exclusion of predation as a factor, the short time period involved, or the somewhat small sample sizes used. The mark-recapture experiments would seem to be the most natural and definitive test of differential survival since the crayfish were exposed to all sources of mortality in a natural setting. However, the lack of differential recapture rates applies only to one location, Lake Kathleen, and could be biased by differential detection by the human observers, and may not be translatable into differential survival rates. We do not feel that differential detectability was an important factor as we regularly observed many brown-morph individuals of *Orconectes virilis* and *O. propinquus* during our searches that were much smaller than the size range we marked, but the potential for bias (seeing the contrasting blue morphs more readily) is present. Differential recapture may not mean differential survival if movement patterns by morphs differ

or movement patterns by crayfish familiar with an area differ from that of introduced individuals. The latter is a potential problem with any transfer experiment. The potential problem of stress related to transport to a new location does not seem important given the direction of the differential in survival, *i.e.*, greater survival by the transferred individuals. A possible reason for the lack of a differential in survival could be the size of crayfish we marked (of both morphs). The average sizes we marked are larger than almost all the crayfish used by Stein and Magnuson (1976) in their studies of bass predation and thus our animals may simply have been too big for predation to be an important factor. Despite these qualifications, the results obtained thus far do not provide confirmation of a function for the colors observed in different habitats.

Behavioral differences.—The behavior patterns tested in the two morphs could interact with color differences in determining survival rates in nature. Responses to disturbance pheromones are thought to function in facilitating avoidance of stressful situations (Hazlett, 1985). Our results suggest no difference between the morphs in this aspect of survival-related behaviors. The level of general activity of animals is often positively correlated with predation risk (Werner and Anholt, *in press*). The lack of a reduced survival rate by blue morphs in Lake Kathleen, which are more active in the laboratory, suggests no such relationship for crayfish in the size range marked.

The fact that blue morphs in the laboratory spent more time with the chelipeds (and whole body) in the raised position (a position used by crayfish when threatened by predators) could have contributed to their higher survival rate in Lake Kathleen. The laboratory data indicating a higher level of general activity of blue morphs and a weaker tendency of blue morphs to move to darker areas of the environment could have contributed to more frequent recaptures in the mark-recapture field study.

General discussion.—Some experimental studies have demonstrated a function for color differences such as Hebert's (Hebert and Emery, 1990) work on melanin concentration in *Daphnia*. Their experiments showed that ultraviolet radiation is a significant source of mortality under some conditions and that pigmentation served to reduce that mortality. This function of color would not appear to apply in the case of these crayfish given the pattern of survivorship in the transfer experiments.

In other cases, functions for color differences have been suggested but not experimentally demonstrated. Bauer (1981) noted that the color patterns of several species of Hippolytid shrimp matched their respective backgrounds and probably served to reduce predation. Both Kent (1901) and Beingesser and Copp (1985) hypothesized a similar function for crayfish color patterns. Experimental evidence to verify this function was not provided in any of these studies. In fact, the only other study addressing possible function from an experimental viewpoint (Momot and Gall, 1971), found no difference in apparent survivorship of the color morphs of *Orconectes virilis* in a lake.

The fact that the colors of crayfish are at least in part a result of environmental induction does not decrease the importance of the question of a functional basis. The ability to respond to some environmental feature could be labelled an "adaptation" just as clearly as any other trait, provided there is a function for the colors resulting from that ability.

Given our results, we cannot rule out the alternative that the colors do not serve any function, at least in the environments we are examining. However, other experiments are planned and other behavioral systems (aggressive interactions, sexual selection) that could be involved and operate differently in different habitats will be investigated.

Acknowledgments.—We wish to thank the staff of the University of Michigan Biological Station for their assistance in these studies. Financial support during these studies was supplied to Robert Thacker

from a Regents' Fellowship from the University of Michigan and a research grant from the UMBS and to Laura Esman, Craig Stafford and Troy Keller from Research Experience for Undergraduates grants from the National Science Foundation to the Biological Station. George Estabrook wrote portions of the PASCAL programs used in the simulations.

LITERATURE CITED

- BAUER, R. T. 1981. Color patterns of the shrimps *Heptacarpus pictus* and *H. paludicola* (Carida: Hippolytidae). *Mar. Biol.*, **64**:141-152.
- BEINGESSER, K. R. AND N. H. COPP. 1985. Differential diurnal distribution of *Procambarus clarkii* (Girard) juveniles and adults and possible adaptive value of color differences between them (Decapoda, Astacidea). *Crustaceana*, **49**:164-172.
- BLACK, J. B. 1974. Inheritance of the blue color mutation in the crayfish *Procambarus acutus acutus* (Girard). *Proc. La. Acad. Sci.*, **38**:25-27.
- DUNHAM, D. W., S. D. JORDAN AND M. NIGLAS. 1979. A new color morph of the crayfish *Orconectes propinquus* (Girard). *Am. Midl. Nat.*, **102**:384-387.
- GANNON, J. E. AND M. W. PADDOCK. 1974. Investigations into ecological and sociological determinants of land-use decisions—a study of inland lake watersheds in northern Michigan. *UMBS Tech. Rep. No. 1*, University of Michigan Biological Station, Pellston. 276 p.
- GHIDALIA, W. 1985. Structural and biological aspects of pigments, p. 301-394. In: D. Bliss and L. H. Mantel (eds.). *The biology of crustaceans*, Vol. 9. Integuments, pigments and hormonal processes. Academic Press, New York.
- HAIRSTON, N. G., JR. 1979. The effect of temperature on carotenoid photoprotection in the copepod *Diaptomus nevadensis*. *Comp. Biochem. Physiol. A*, **62**:445-448.
- HAZLETT, B. A. 1966. Social behavior of the Paguridae and Diogenidae of Curacao. *Stud. Fauna Curacao Other Caribb. Isl.*, **23**:1-143.
- . 1985. Disturbance pheromones in the crayfish *Orconectes virilis*. *J. Chem. Ecol.*, **11**:1695-1711.
- . 1989. Additional sources of disturbance pheromone affecting the crayfish *Orconectes virilis*. *J. Chem. Ecol.*, **15**:381-385.
- . 1990. Source and nature of disturbance-chemical system in crayfish. *J. Chem. Ecol.*, **16**:2263-2275.
- , D. RITTSCHOF AND D. RUBENSTEIN. 1974. Behavioral biology of the crayfish *Orconectes virilis* I. Home range. *Am. Midl. Nat.*, **92**:301-319.
- HEBERT, P. D. N. AND C. J. EMERY. 1990. The adaptive significance of cuticular pigmentation in *Daphnia*. *Funct. Ecol.*, **4**:703-710.
- HERRICK, F. H. 1895. The American lobster. A study of its habits and development. *Bull. U.S. Fish Comm.*, **15**:1-252.
- KENT, W. J. 1901. The colors of the crayfish. *Am. Nat.*, **35**:933-936.
- KORNERUP, A. AND J. H. WANSCHER. 1983. *Methuen handbook of colour*, 3rd ed. Methuen, London. 251 p.
- MANLY, B. F. J. 1991. *Randomization and Monte Carlo methods in biology*. Chapman and Hall, New York. 281 p.
- MATHER, M. E. AND R. A. STEIN. In Press. Using growth mortality tradeoffs to explore a crayfish species replacement in streams, riffles and pools. *Canad. J. Fish. Aquatic Sci.*
- MOMOT, W. T. AND J. E. GALL. 1971. Some ecological notes on the blue color phase of the crayfish, *Orconectes virilis*, in two lakes. *Ohio J. Sci.*, **71**:363-370.
- AND H. GOWING. 1977. Response of the crayfish *Orconectes virilis* to exploitation. *J. Fish Res. Board Can.*, **34**:1212-1219.
- NEWCOMBE, C. L. 1929. A new blue crayfish. *Science*, **70**:217.
- PENN, G. H., JR. 1951. A color anomaly of the Louisiana red crayfish, *Procambarus clarkii* (Girard). *Proc. La. Acad. Sci.*, **14**:66-67.
- PORTMANN, A. 1959. *Animal camouflage*. Univ. Mich. Press, Ann Arbor. 111 p.
- RA'ANAN, Z. AND A. SAGI. 1985. Alternative mating strategies in males of the freshwater prawn *Macrobrachium rosenbergii* (de Man). *Biol. Bull.*, **169**:592-601.

- RINGELBERG, J. 1980. Aspects of red pigmentation in zooplankton, especially copepods, p. 91-97. In: W. C. Kerfoot (ed.). Evolution and ecology of zooplankton communities. Univ. Press New England, Hanover, N.H.
- ROBERTS, T. W. 1944. Light, eyestalk chemical, and certain other factors as regulators of community activity for the crayfish, *Cambarus virilis* Hagen. *Ecol. Monogr.*, **14**:360-389.
- SMILEY, J. W. AND W. W. MILLER. 1971. The occurrence of blue specimens of the crayfish *Procambarus acutus acutus* (Girard) (Decapoda, Astacidae). *Crustaceana*, **20**:221.
- STEIN, R. A. AND J. J. MAGNUSON. 1976. Behavioral response of crayfish to a fish predator. *Ecology*, **57**:751-761.
- STEVENSON, J. R. 1985. Dynamics of the integument, p. 2-42. In: D. E. Bliss and L. H. Mantel (eds.). The biology of crustaceans, Vol. 9. Academic Press, New York.
- WALD, G. 1968. Single and multiple visual systems in arthropods. *J. Gen. Physiol.*, **51**:125-156.
- WERNER, E. E. AND B. R. ANHOLT. 1992. Ecological consequences of the trade off between growth and mortality rates mediated by foraging activity. *Am. Nat.*, in press.
- WETZEL, R. G. AND G. E. LIKENS. 1991. Limnological analyses, 2nd ed. Springer-Verlag, New York. 391 p.

SUBMITTED 12 MARCH 1992

ACCEPTED 24 JULY 1992