# NOTES AND NEWS

## SALINITY TOLERANCE IN COLOR PHASES OF FEMALE GREEN CRABS, *CARCINUS MAENAS* (LINNAEUS, 1758)

BY

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### INTRODUCTION

Despite its common name, the green crab *Carcinus maenas* (Linnaeus, 1758) ranges from green through orange to red coloration on the ventral surface. In the literature, crabs have been lumped into two color groups, green and red (Reid et al., 1997). For the rest of this paper, green crabs will refer to any *C. maenas*, while green phase and red phase will refer to individuals of each color category. In male crabs, the red coloration, which develops during prolonged intermolt, is associated with several ecological and physiological changes in the crabs (see Reid et al., 1997; Styrishave & Andersen, 2000 for reviews).

Red phase crabs are more common in subtidal habitats than in intertidal or estuarine, in both European (Crothers, 1968; McGaw & Naylor, 1992a; Hunter & Naylor, 1993; Reid et al., 1997) and New England (McKnight et al., 2000) populations, in a large part due to their narrower physiological tolerances when compared to green phase crabs (Reid & Aldrich, 1989; McGaw & Naylor, 1992a, b). Red phase individuals have thicker carapaces, more robust chela muscles, and are stronger than similar-sized green phase individuals (Kaiser et al., 1990). These size and strength differences result in higher mating success for red phase males when compared to green phase males, in both field and laboratory experiments (Reid et al., 1997).

A trade-off exists between mating success and physiological tolerance. Reid et al. (1997) suggest that the color phases are part of an evolutionary stable

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strategy where a choice is made between molting and not molting. Crabs who choose to molt benefit by increasing their size and retaining wide physiological tolerance. Non-molters, on the other hand, suffer decreased physiological tolerance but improve their chances of mating. Developing the idea further, Wolf (1998) suggests that in male crabs the two color phases may represent different parts of a crab's life cycle, with the green phase being a period of growth with frequent molting and the red phase a period of maximum reproductive effort with prolonged intermolt periods.

With rare exception (Stryrishave & Andersen, 2000), all work on color phases in *Carcinus maenas* has tested only male crabs. Females are different from males: morphologically, ecologically, and behaviorally. Females tend to have smaller chelae (Crothers, 1967), to forage less actively, and to migrate less extensively during high water (Hunter & Naylor, 1993), than similar-sized males. Females have different molting constraints. They mate immediately after molting. In addition, females do not molt while carrying eggs (Crothers, 1968). Finally, red phase females are more commonly collected than red phase males (McGaw & Naylor, 1992b; McKnight et al., 2000) and some females have retained red coloration through the molt in the laboratory (Reid et al., 1997).

In order to understand the ecological role of color change in *Carcinus maenas*, much more information is needed, particularly on the physiology and ecology of color change in female crabs. We designed this study to determine if red and green phase female *C. maenas* exhibit the same tolerance differences previously reported for male crabs. Our specific objectives were to compare survivorship between red and green phase females, and to examine osmoregulation ability in red and green phase females exposed to a range of salinities.

## METHODS

For survival tests, crabs were collected by hand at Pleasure Beach in Waterford, Connecticut or were obtained from the Millstone Nuclear Power Station Environmental Lab in Niantic, CT. In all, 25 green females and 39 red females between 40 and 59 mm carapace width were tested over the course of three trials. In each trial, females of each color phase were placed into one of two experimental tanks, each holding approximately 20 liters of 10% seawater. In addition, females (10) of each color phase were placed in a 40 liter control aquarium of ∼100% seawater. Water temperature varied with room temperature, between 25 and 28$^{o}$ Celsius. Experimental tanks were inspected every 2-3 hours for a period of 3 days, or until all crabs were dead. Results for all green phase females and all red phase females were pooled and survival curves for red and green phase females were compared

TABLE I

Number of individuals of *Carcinus maenas* (L.) in each salinity trial. Due to mortality, a different number of green and red phase crabs completed the trial in each salinity. These crabs are the crabs included in fig. 2

| External salinity (mOsm) | Number of green females | Number of red females |
|---|---|---|
| 1336 | 2 | 4 |
| 1050 | 2 | 4 |
| 923 | 1 | 1 |
| 871 | 1 | 1 |
| 825 | 1 | 1 |
| 620 | 2 | 3 |
| 600 | 2 | 3 |
| 441 | 1 | 1 |

using product-limit survival estimates (Kaplan-Meier method, see Krebs, 1989), and tested for significant differences using a Wilcoxon $\chi^2$ test.

For the osmoregulation tests, crabs were collected and held in 100% seawater for several days prior to testing. If crabs dropped limbs, died, or became lethargic during the experiment, those individuals were discarded. Since the data are comparative, if at least one crab from each group did not survive at least 12 hours in the experimental salinity, data from both groups were discarded. Data are reported from 30 animals, 12 green phase females and 18 red phase (see table I for list of salinities and the number of individuals tested in each trial). More red phase crabs were included in each trial due to expected differences in mortality between the phases. Hemolymph was tested prior to immersion and then again at least 12 hours after immersion by drawing hemolymph samples (less than 0.2 ml) through the membrane in claws or walking legs. Samples were tested in a vapor pressure osmometer (Wescor 5100 series). In preliminary experiments there was no significant difference between 12 and 24 hour hemolymph measurements in individual crabs (Mann-Witney U test: $n = 7$; $Z = -0.319$; $P = 0.7494$). Several crabs included here were also tested 6 hours post-immersion, but the multiple sampling was discontinued in later trials to minimize mortality at extreme salinities. In all trials, red phase and green phase females in the same trial were treated with the same protocol. Significant differences between slopes and elevations were tested using ANCOVA.

## RESULTS

Green phase females survived significantly longer than red phase females (green phase females: average time $= 28.45 \pm 1.318$ hours; red phase females: average

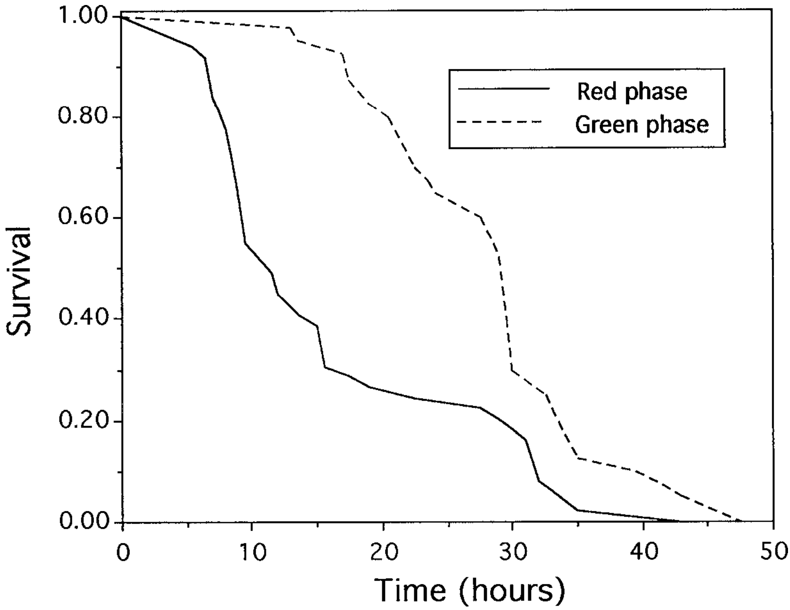


Fig. 1. Product-limit survival curves for green and red phase females. Each curve represents the number of female *Carcinus maenas* (L., 1758) remaining alive each day of the experiment.

time = 16.07 ± 1.48 hours; $\chi^2 = 15.08$, $P < 0.0001$) (fig. 1). No control animals died during the experiment.

Crabs of both phases osmoregulate well (fig. 2) in the range of salinities tested. If the crabs were conforming to external salinities, the relationship between hemolymph osmolality and external osmolality would approximate 1.0. This is not the case for either green phase or red phase females in our study. For green phase females $Y = 0.571X + 513$; $r^2 = 0.916$, and for red phase females $Y = 0.652X + 394.731$; $r^2 = 0.947$. At salinities below full strength seawater, crabs maintained their hemolymph osmolality above external osmolality. Green phase females, however, maintained their hemolymph osmolalities significantly higher than did red phase females (ANCOVA for homogeneity of slopes: $F = 1.58977$, $P = 0.2186$; for homogeneity of elevations $F = 6.3621$, $P = 0.0179$).

## DISCUSSION

Our work confirms that female crabs exhibit color-related differences in salinity tolerance like those of male crabs. Red phase females are less tolerant of low salinity and do not osmoregulate as well as green phase females. Differences in physiological tolerance and morphology between color phases of *Carcinus maenas* have been well established for male crabs (Reid et al., 1997), as has the ecological

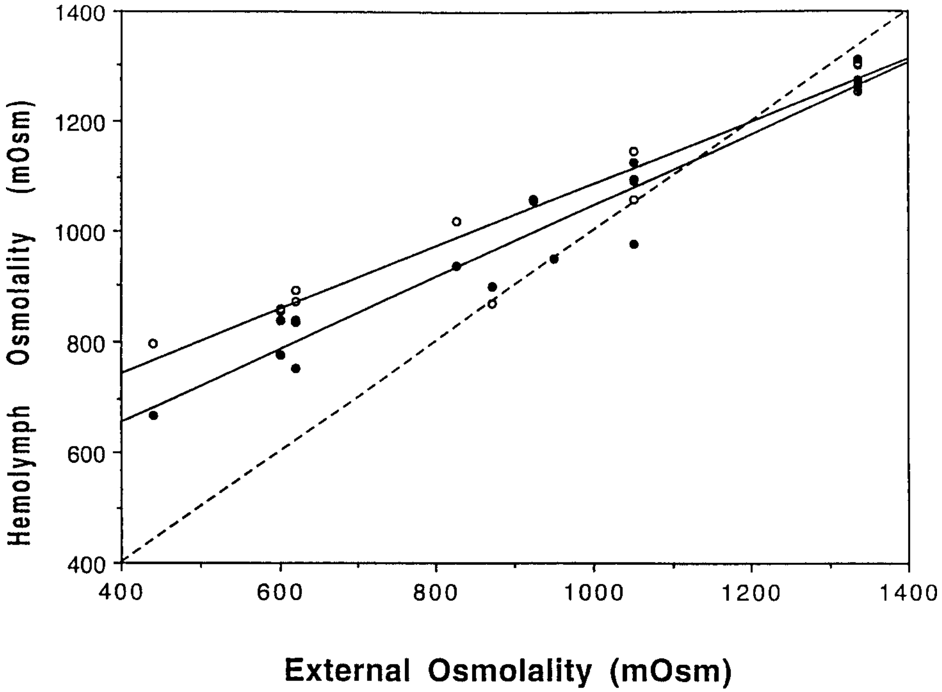


Fig. 2. Hemolymph osmolality curves for red and green phase females of *Carcinus maenas* (L., 1758). On the *y*-axis are the hemolymph osmolalities after at least 12 hours exposure to the experimental salinity. On the *x*-axis are the experimental salinities. Each open circle represents the hemolymph osmolality for an individual green phase female. Each closed circle represents the hemolymph osmolality for an individual red phase female. A circle representing one individual may be obscured by the circle representing another individual if hemolymph values are similar.

importance of the phases (Wolf, 1998). Red color represents a period of maximum reproductive effort and green color a period of maximum growth. Little is known about the phenomenon in female crabs, which exhibit the same color morphology.

This study, however, is only a first step in examining color phases in female crabs. There are many differences between the sexes including maximum size, molt frequency, and distribution on the shoreline, that may influence or be influenced by color phase. Molt frequency or schedule may cause sex-related differences in coloration. Color variation in males is related to the molt cycle; red coloration appears during an extended intermolt. Females, however, may follow a different molting schedule than males (Crothers, 1967), causing seasonal differences in color phase distribution. Styrishave & Andersen (2000) for example found significant differences in hepatopancreas fatty acid profiles between male and female crabs.

Indeed, green phase females were rare in our early summer collections, and most of the females present were dark red in coloration. Red phase males, in contrast, were rare in most early summer collections, becoming more abundant later in the summer (McKnight, 1999). In our collections, green phase males have always been present (McKnight, 1999; McKnight et al., 2000). The occasional rarity of green phase females indicates that either virtually all females in the sampled population undergo prolonged intermolt, or that females do not always lose red coloration upon molting. Such color retention has reportedly been observed in the laboratory (Reid et al., 1997).

Given the differences between male and female *Carcinus maenas*, there is not enough information to speculate on the ecological significance of coloration in female crabs. However, the differences in physiological tolerance between color morphs in male crabs, at least with respect to salinity tolerance, are also present in females, confirming that red coloration is physiologically similar in males and females.

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