

RESEARCH ARTICLE

Effects of molting on the visual acuity of the blue crab, *Callinectes sapidus*

Jamie Baldwin* and Sönke Johnsen

Department of Biology, Box 90338, Duke University, Durham, NC 27708, USA

*Author for correspondence (jlb54@duke.edu)

Accepted 20 May 2011

SUMMARY

In crustaceans with compound eyes, the corneal lens of each facet is part of the exoskeleton and thus shed during molting. Here we used an optomotor assay to evaluate the impact of molting on visual acuity (as measured by the minimum resolvable angle, α_{\min}) in the female blue crab, *Callinectes sapidus*. We found that visual acuity decreases substantially in the days prior to molting and is gradually recovered after molting. Four days prior to molting, α_{\min} was 1.8 deg ($N=5$), a value approximating the best possible acuity in this species. In the 24 h before ecdysis occurred, α_{\min} increased to 15.0 deg ($N=12$), corresponding to an eightfold drop in visual acuity. Within 6 days after molting, α_{\min} returned to the pre-molting value. Micrographs of *C. sapidus* eyes showed that a gap between the corneal lens and the crystalline cone first appeared approximately 5 days prior to shedding and increased in width as the process progressed. This separation was likely responsible for the loss of visual acuity observed in behavioral tests. In blue crabs, mating is limited to the period of the female's pubertal molt, and a reduction in acuity during this time may have an effect on the sensory cues used in female mate choice. The results described here may be broadly applicable to all arthropods that molt and have particular importance for crustaceans that molt multiple times in their lifetime or have mating cycles that are paired with molting.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/18/3055/DC1>

Key words: blue crab, molting, vision.

INTRODUCTION

The visual systems of the nearly 50,000 extant crustacean species are highly variable. Most crustaceans have compound eyes, with minimum inter-ommatidial angles varying from approximately 50 deg in *Daphnia* (Land and Nilsson, 1990) to approximately 1 deg in certain stomatopods (Land, 1999). Although some crustaceans are monochromats, color vision is common, in some cases involving as many as 12 distinct channels (reviewed by Cronin, 2006). Overall, crustaceans can be highly visual creatures, using vision to find food and shelter and avoid predation. Vision is also used in both agonistic and sexual communication (Schöne, 1968; Molenock, 1975; Bruski and Dunham, 1987; Christy and Salmon, 1991; Detto et al., 2006; Chiou et al., 2008).

Molting is a feature common to all crustaceans, and this process may create a visual challenge that has not yet been examined. In crustaceans with compound eyes, the multi-faceted lenses are composed of modified exoskeleton (Land and Nilsson, 2002). When a crustacean molts, the entire exoskeleton is shed, including the faceted lenses. Prior to molting, the exoskeleton separates from the underlying hypodermis, which then secretes a new epicuticle (Smith and Chang, 2007). The outer exoskeleton then ruptures, the animal extracts itself from its old cuticle and the body quickly expands its new cuticle *via* rapid water intake (Smith and Chang, 2007). Calcification of the new exoskeleton begins within 12 h of molting and continues for up to 30 days after molting (Dendinger and Alterman, 1983; Cameron, 1989). Thus, prior to molting, the existing lenses of each ommatidium must be partially resorbed, resulting in a separation of the lenses from the underlying tissue. Following this separation, the underlying tissue secretes new lenses. This process introduces a cavity between the old and new lenses,

and the secretion of the new cuticle temporarily adds an additional tissue layer in the eye. These changes increase the distance between the lenses and the photoreceptors and possibly disrupt the light paths in the eye, either of which would affect visual acuity.

The blue crab, *Callinectes sapidus* Rathbun 1896, has large, stalked apposition compound eyes with a minimum inter-ommatidial angle of approximately 1.5 deg (Eguchi and Waterman, 1965; Land and Nilsson, 2002). Blue crabs are often thought to simply act as scavengers, but they are excellent visual predators, reportedly using vision to catch fish, stalk fiddler crabs and even bury themselves up to their eyes to ambush a prey item (Abbott, 1967; Hines, 2007). Behavioral experiments testing visual responses to objects of various shapes and colors suggest that *C. sapidus* is able to discern blue, yellow and red (Bursey, 1984; Baldwin and Johnsen, 2009).

Vision also plays an important role in blue crab communication, with a number of visual postures being used in agonistic (Jachowski, 1974) and courtship behaviors (Teytaud, 1971). The male blue crab can visually choose mates and prefers females with red claws over those with claws of other hues (Baldwin and Johnsen, 2009), and female blue crabs may use visual cues in identifying males (Teytaud, 1971). Because females pair with mates just prior to molting (Jivoff and Hines, 1998), possible changes in visual acuity during this period could affect females' use of vision in mate choice.

In the present study, we investigated changes in blue crab visual acuity during the molting process using behavioral and morphological assays. Using the optomotor response, an innate reflex that stabilizes the visual field, we evaluated blue crab visual acuity during molting. In addition, eyes from individuals undergoing molting were examined for changes in structure.

MATERIALS AND METHODS

Study species

Blue crabs were collected in April 2010 from Jarrett Bay, near Smyrna, NC, USA (34°45'31"N, 76°30'44"W). Portunid crabs are, in part, characterized by having the fifth pair of walking legs flattened into swimming paddles. In the blue crab, a visual examination of these paddles can reveal signs of an impending molt, such as a separation between the existing exoskeleton and the underlying tissue [see fig. 2 in Smith and Chang (Smith and Chang, 2007)]. Thus, visual inspection of the paddle is used by crab fishermen to identify premolt crabs, and changes in the appearance of the blue crab paddle can be used to estimate the number of days prior to an individual's molt. Twelve female crabs approaching their terminal, adult molt and predicted to molt within 5 days were collected, transported to Durham, NC, and kept in individual compartments in a recirculating seawater system at a temperature of 22–24°C and a salinity of 28–30‰ on a 12 h:12 h light:dark cycle. Pieces of shrimp were offered to crabs daily throughout the trials, though crabs only fed after molting.

Optomotor response

The average visual acuity of the eye can be determined *via* stereotypical behaviors such as the optomotor response, which can be elicited by placing an animal inside a rotating black-and-white striped drum (Fig. 1). The animal reflexively turns or moves its eyes in the same direction as the rotation of the stripes, thereby stabilizing its visual field (Reichardt, 1961; McCann and MacGinitie, 1965). This response can be used to determine an animal's visual acuity (assessed *via* the minimum resolvable angle, α_{\min}) by finding the angular width of the stripe pattern that the animal no longer responds to. It should be noted that, although visual acuity in this study is assessed and quantified by the minimum resolvable angle, this parameter is the inverse of visual acuity. In other words, a decrease in α_{\min} corresponds to an increase in visual acuity.

Our optomotor apparatus consisted of a 56 cm diameter drum that was rotated from below by a computer-controlled stepper motor (STP-MTRH-23079, AutomationDirect, Atlanta, GA, USA). Stripe patterns, with angular widths of 1.4, 1.6, 1.8, 2.1, 2.3, 2.9, 3.6, 4.8, 6.2, 12, 14, 32 and 48 deg for each pair of one black and one white stripe (as viewed from the center of the drum), were printed using a laser printer on standard printer paper and mounted within the drum. During testing, crabs were placed within a water-filled 30 cm diameter cylindrical acrylic tank suspended inside the striped drum

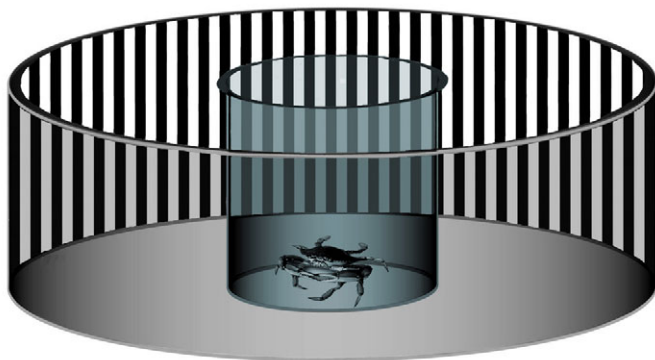


Fig. 1. Diagram illustrating the optomotor apparatus. A test subject is placed in a cylindrical acrylic tank suspended inside a striped drum that is mounted to a computer-controlled stepper motor. Overhead lighting illuminates the arena and crab behavior is observed *via* a video camera.

and observed using a video camera mounted overhead. The positions of the crabs were noted during experiments, and the perceived angular widths of the stripe patterns were calculated based on the shortest distance between the eyes and the pattern. Although calculations of the angular widths would have been simplified by confining subjects to a smaller test arena, or by tethering individuals, this species did not respond in preliminary tests when confined to a smaller tank. A uniform 50% gray control pattern was also prepared and used during experiments. The test arena was illuminated by an overhead incandescent lamp fitted with a diffuser, resulting in a downwelling irradiance of 8.6×10^{14} photons $\text{cm}^{-2} \text{s}^{-1}$ (integrated from 400 to 700 nm), which is similar to irradiance levels that have been used in previous *C. sapidus* behavior experiments (Baldwin and Johnsen, 2009).

The 12 premolt female blue crabs were tested daily for 4 to 5 days prior to molting and for 6 days after molting. One individual perished during molting, so no postmolt data were recorded for it. Prior to the beginning of the experiment, individuals were placed in the test arena and allowed to acclimate for 15 min. The drum was then rotated at a constant speed of 2 revolutions min^{-1} , an optimal speed determined in preliminary experiments with this species. If the crab responded by walking in the direction of the rotation, the drum was counter-rotated to ensure that the crab was moving in response to the stripes. During initial tests, individuals were first tested with narrow stripes, moving to wider stripes as needed to elicit a response. In successive trials, individuals were tested beginning with the narrowest stripe pattern that stimulated a response in the previous day's test. If the crab responded, it was tested with increasingly narrower stripes until it failed to respond. If the crab did not respond, wider stripe patterns were tested. This approach minimized the amount of time that crabs were exposed to the testing conditions. In the first 3 days of testing, each crab was subjected to the gray control once during its testing period. In subsequent days, each individual was tested with the gray control every other day. The gray control was tested a total of 82 times and did not elicit a response in any individual.

Analysis of optomotor data

The individuals used in the optomotor assays were wild-caught crabs. Although we strived to collect specimens that were at approximately the same molting phase, there was unavoidable variation in the number of days each crab was from molting. Thus, the results were aligned by molting time. This decreased the *N*-value for days four and five prior to molting, because not all crabs were 4 or 5 days away from molting at the time of capture. We described the data using median and interquartile range, rather than mean and standard error, because the presence of outliers skewed the data far from a normal distribution. We compared the α_{\min} at each day to that found 4 days prior to molting, as these values are similar to estimates of α_{\min} during intermolt phases. The non-parametric Mann–Whitney *U*-test was used because the distributions were not normal. The statistical issues inherent in multiple testing were regulated using the Benjamini and Hochberg procedure, which controls for false discovery rate (Benjamini and Hochberg, 1995).

Eye morphology

Eyes were surgically excised from crabs at the Hooper Family Seafood shedding operation in Smyrna, NC, in May 2010. Using the same methods described above, premolt crabs were identified and grouped by molt stage. Eyes were taken from intermolt crabs, crabs molting within 3–5 days, crabs molting within 1–3 days, molting crabs and postmolt crabs no more than 2 h after molting. Eyes were fixed in

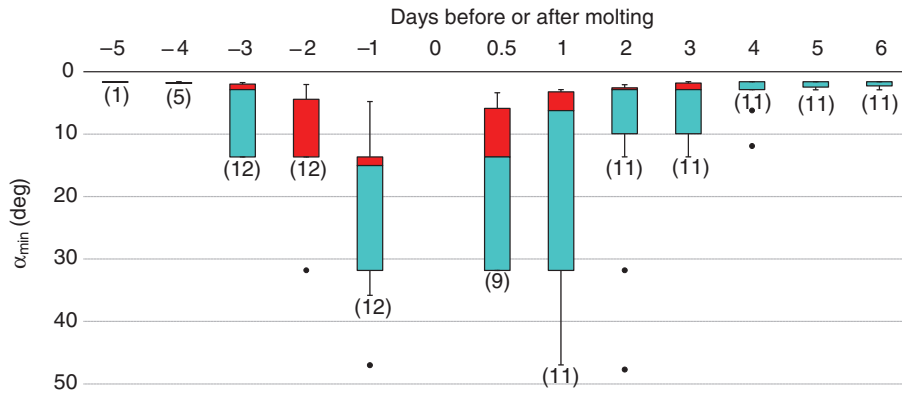


Fig. 2. Box-and-whisker plot showing the minimum resolvable angle, α_{\min} , before, during and after molting. The red bars are bounded by the lower (25th percentile) quartile and the median. The blue bars are bounded by the median and the upper (75th percentile) quartile. The error bars encompass the minimum and maximum of the data, with six outliers (defined as points farther than 1.5 times the interquartile range from the median) plotted as individual points.

4% formaldehyde buffered with artificial seawater for 48 h and then stored in 70% ethanol. Twenty-four hours prior to sectioning, eyes were rinsed and stored in phosphate buffered saline. Eyes were then frozen and bisected horizontally across the long axis using a cryostat. One half of each eye was examined and photographed under a stereoscopic dissecting microscope (Lumar V12, Zeiss Inc., Jena, Germany). Dimensions of the structures in the eye and the gap between the corneal lens, crystalline cone and newly secreted lens were measured using the AxioVision software (version 4.6.1.0, Zeiss Inc.), a program that controlled the microscope and was calibrated according to the installed objectives and distance from the sample. In eyes that had variable separation, we measured the size of the gap at a location that appeared to be between the two extremes, with the intention of measuring the average separation. Four eyes were measured and analyzed at each stage.

RESULTS

Optomotor response

A significant loss in visual acuity was documented in *C. sapidus* in the days prior to molting (Fig. 2; Table 1; supplementary material Fig. S1). Four days prior to molting, the median α_{\min} was 1.8 deg. In the 24 h prior to molting, the median α_{\min} rose to 15.0 deg. After molting, visual acuity improved daily, with the median α_{\min}

dropping to 1.6 deg within 6 days. Recovery of normal visual acuity was usually achieved within 6 days of molting, although by 3 days after molting the median α_{\min} of 2.9 deg was not significantly different from that measured 4 days prior to molting. There was substantial variation in individual acuity and in the rate of vision loss and recovery, with a few outliers having very poor visual acuity during molting (Fig. 2).

Morphology of eyes during molting

We found that molting affected the structure of blue crab eyes (Fig. 3). In the intermolt crab eye, no gap between the corneal lens and underlying tissue was apparent. Eyes from crabs molting within 3–5 days displayed a gap of approximately $21.3 \pm 9.8 \mu\text{m}$ (mean \pm s.e.m., $N=4$) between the corneal lens and the crystalline cone. The gap increased to $68.8 \pm 34.4 \mu\text{m}$ ($N=4$) in the eyes of crabs predicted to molt within 1–3 days. These eyes also showed a thin, faceted layer over the crystalline cone that was likely the newly secreted lens. In molting crabs, the photographs show the eye as it was exiting the exoskeleton. The mean spacing between the eye and the outer lens was $369 \pm 221 \mu\text{m}$ ($N=4$). In the postmolt eyes, the eyes were taken from soft crabs less than 2 h after molting and were not yet fully expanded. So, at the time of fixation, they were malformed and wrinkled.

Table 1. Individual results of optomotor response tests showing the minimum resolvable angle, α_{\min} (deg), before, during and after molting of the blue crab, *Callinectes sapidus*

Individual	Days before or after molting													
	-5	-4	-3	-2	-1	0	+0.5	+1	+2	+3	+4	+5	+6	
1	—	1.8	2.1	13.6	13.6	—	4.8	4.1	2.9	1.6	1.6	1.6	1.6	
2	1.6	1.8	13.6	31.8	35.8	—	6.2	2.9	2.9	2.9	1.6	1.6	1.6	
3	—	1.6	2.1	4.8	31.8	—	3.4	2.9	2.3	1.6	1.6	1.6	1.6	
4	—	—	2.9	13.7	23.9	—	—	47.0	47.8	13.6	6.32	2.9	2.9	
5	—	—	13.6	13.6	16.4	—	—	31.8	13.6	13.6	11.9	2.9	2.5	
6	—	—	1.8	2.1	4.8	—	31.8	13.6	2.9	2.9	2.9	2.9	2.9	
7	—	—	2.9	2.3	13.6	—	31.8	31.8	6.2	6.2	2.1	2.1	2.1	
8	—	—	4.8	13.6	31.8	—	—	6.2	3.6	2.1	1.6	1.6	1.6	
9	—	—	13.6	13.6	47.0	—	13.6	3.6	2.1	2.1	1.6	1.6	1.6	
10	—	1.8	1.8	13.6	13.6	—	—	—	—	—	—	—	—	
11	—	—	1.8	3.4	13.6	—	31.8	31.8	31.8	13.6	2.9	2.1	2.1	
12	—	1.8	13.6	13.6	13.6	—	13.6	2.9	2.1	1.6	1.6	1.6	1.6	
Median	1.6	1.8	2.9	13.6	15.0	—	13.6	6.2	2.9	2.9	1.6	1.6	1.6	
Lower (25th) quartile	1.6	1.7	2.0	4.4	13.6	—	5.9	3.2	2.6	1.8	1.6	1.6	1.6	
Upper (75th) quartile	1.6	1.8	13.6	13.6	31.8	—	31.8	31.8	9.9	9.9	2.9	2.5	2.3	
Mean \pm s.e.m.	1.6	1.7 \pm 0.03	6.2 \pm 1.6	11.7 \pm 2.4	21.7 \pm 3.6	—	17.1 \pm 3.0	16.2 \pm 4.9	10.7 \pm 4.6	5.6 \pm 1.8	3.2 \pm 1.0	2.0 \pm 0.2	2.0 \pm 0.18	
P	—	—	0.027*	0.002*	0.002*	—	0.003*	0.002*	0.002*	>0.2	>0.2	>0.2	—	

Molting is time zero. The median and interquartile ranges are reported for each time point. The probability that α_{\min} differs significantly from that of the intermolt crab eye was calculated using the Mann–Whitney U-test. * $P < 0.05$.

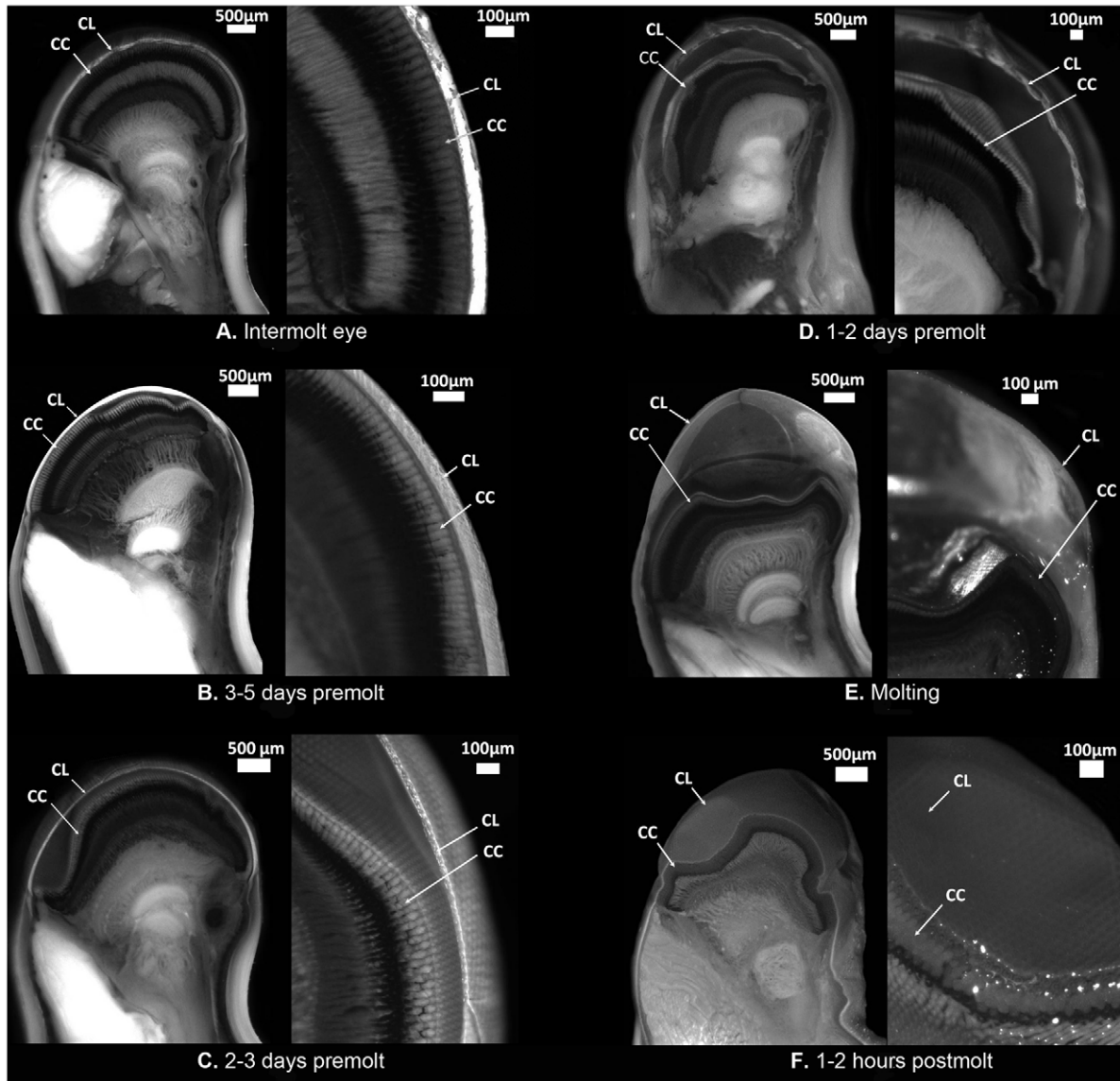


Fig. 3. *Callinectes sapidus* eye before, during and after molting. The left photos in each pair are of halves of entire eyes. The right photos are close-ups that show the relationship between the corneal lens and crystalline cone. (A) Intermolt crab that is at least 14 days away from molting. (B) Crab that will molt in 3 to 5 days. (C) Crab that will molt in 2 to 3 days. (D) Crab that will molt in 1 to 2 days. (E) Crab that was in the process of shedding its exoskeleton. (F) Soft crab immediately after molting. CC, crystalline cone; CL, corneal lens; RB, rhabdom; RC, reticular cell.

DISCUSSION

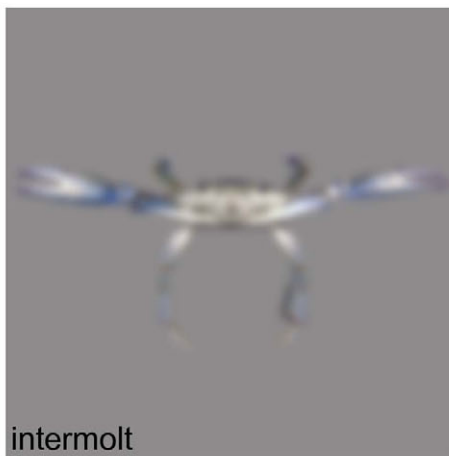
The behavioral results of this study indicate a significant, temporary loss of visual acuity during molting in female blue crabs. Overall, the median values of α_{\min} in *C. sapidus* increased from 1.8 to 15 deg during the molting period, rising as high as 48 deg in certain individuals. Three days prior to and after molting, the median α_{\min} was 2.9 deg, similar to that of the crepuscular dung beetle, *Onitis alexis* (Warrant and McIntyre, 1990). In the 24 h period before molting, the median α_{\min} in individual crabs was 15 deg, with some individuals having visual acuity comparable to that of a planarian flatworm (Land and Nilsson, 2002). Although the duration and degree of change in visual acuity varied among individuals, all demonstrated a clear and substantial decrease (supplementary material Fig. S1). In most individuals, the onset of the decline in acuity occurred 3 days prior to molting. Recovery of visual acuity was also variable, but most individuals recovered normal vision

within 3 to 6 days after molting. The recovery of individuals strongly suggests against any acclimation to the optomotor assay with daily testing. Taken together, our results imply that blue crabs are faced with approximately 6 to 9 days of reduced visual acuity during the molting period (Fig. 4).

Our examination of the eyes of molting blue crabs showed that the morphology of a molting eye differs from that of a typical intermolt apposition compound eye, and these differences could be responsible for the loss of visual acuity observed during molting. In the typical apposition compound eye, each individual ommatidium functions independently, sampling light from its visual field (Land, 1997). Incoming light is focused through the corneal lens onto the distal tip of the rhabdom (Fig. 5A) (Land and Nilsson, 2002). In molting eyes, the lens separates from the underlying crystalline cone, introducing a gap that ranges from approximately 19 μm in early premolt crabs to approximately 0.5 mm in crabs close



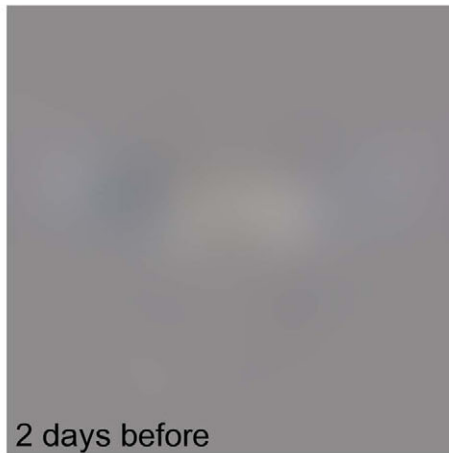
original image



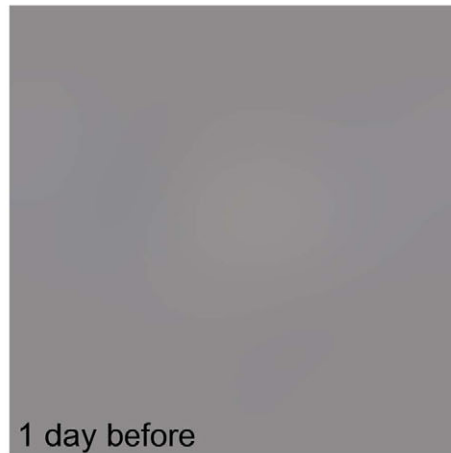
intermolt



3 days before



2 days before



1 day before

Fig. 4. Effects of molting on vision. Simulated appearance of a courting male blue crab as viewed by a female blue crab from a distance of 15 cm. During intermolt periods, the median α_{\min} is approximately 1.6 deg; 3 days, 2 days and 1 day before molting, this value is 2.9, 13.6 and 15 deg, respectively. The images were created by convolving the top image with a Gaussian point spread function that corresponded to a modulation transfer function whose contrast reproduction at the maximum resolvable spatial frequency was less than 2% (the 2% value was chosen because it approximates the minimum contrast threshold for many organisms). The end result of the convolution is an image that contains no detectable spatial information spanning angles less than α_{\min} . The convolution was performed for each color channel separately using standard Fourier methods on 2048×2048 pixel images. See Johnsen et al. (Johnsen et al., 2004) for further details.

to molting (Fig. 5B). The corneal lens of each ommatidium has a set focal distance, so the large separation generated during molting results in the rhabdom receiving a defocused image. In addition, the secretion of the future lens between the crystalline cone and the lens of the existing exoskeleton (Fig. 5C) may further affect the eye's focusing ability by increasing the distance between the lens and the rhabdom and by adding a layer of material with a high refractive index. Aside from changing the focal point, the growing gap may allow light entering one facet of the eye to fall onto multiple ommatidia, disrupting the ability of each ommatidium to sample

light from only its particular field of vision and leading to further loss of acuity.

Further, in the immediate postmolt crab, the eye is structurally weak and misshapen. The postmolt body, and likely the eye, is inflated by the rapid uptake of water that occurs for several hours after molting (Mangum, 1992). Calcification of the new exoskeleton takes several days to weeks (Dendinger and Alterman, 1983; Cameron, 1989). The eye and the corneal lenses likely do not function correctly until inflation of the body and partial hardening of the exoskeleton occur. Reduced visual acuity in recently molted

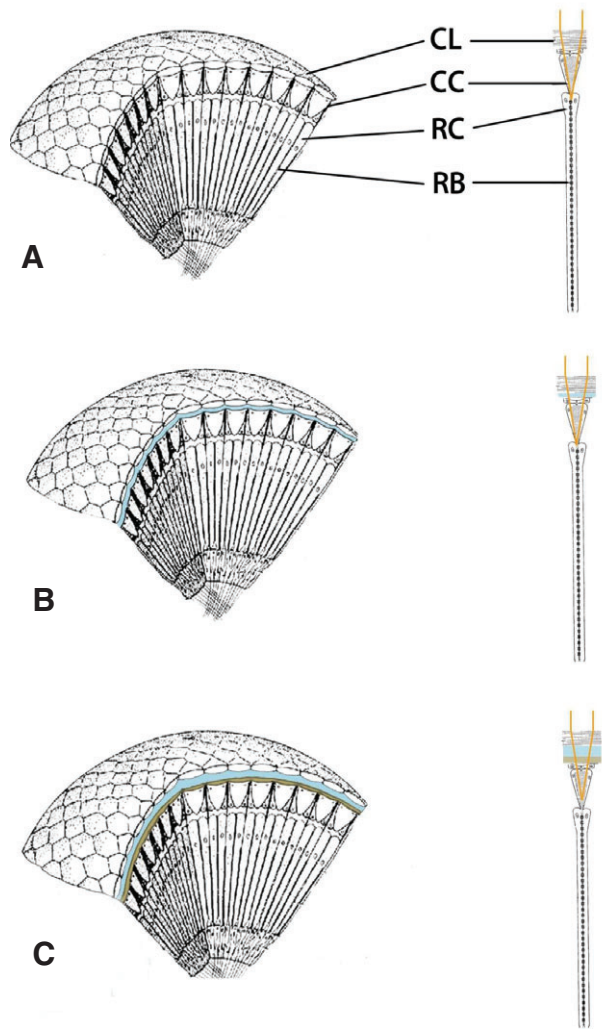


Fig. 5. Diagrams of apposition compound eyes and ommatidia showing the progressive changes in the eye during molting, including the possible light path in the ommatidia (modified from Land and Nilsson, 2002). (A) Intermolt eye. (B) Crab that will molt in 3 to 5 days. (C) Crab that will molt in 1 to 3 days. The growing space between the corneal lens and crystalline cone is shown as a blue layer. The newly secreted cuticle is shown as a tan layer. The orange lines indicate the probable light path and focal position in the eye. CC, crystalline cone; CL, corneal lens; RB, rhabdom; RC, retinular cell.

crabs may be attributed to functional limitations of the soft crab eye.

We acknowledge that there are certain limitations and caveats to both the behavioral and morphological components of our study. One, there is notable individual variation in the results of both components. The variation observed may simply be due to random individual differences in the size of the gap between the lens and the crystalline cone as the molting process occurs. Although variables such as individual or environmental conditions may play a role in an individual's progression through the molt cycle, it is unclear whether these types of factors affect the separation size between the exoskeleton and underlying tissue.

Also, it would be fascinating to pair the behavioral and morphological assays in individual subjects. However, at this time eye morphology cannot be examined without removing the eyes, which would terminate any behavioral assay of vision. Similarly,

in the morphological examination, each crab's progress in the molting cycle was estimated prior to removing and examining the eyes, giving an inexact pairing of eye morphology and molting stage. We could have removed the eyes and kept each individual alive until it molted in order to determine an exact time frame. However, removing the eyes induces physical damage, particularly blood loss, which reduces an individual's survival during ecdysis. Further, we were concerned with the hormonal effects of removing the neuroendocrine X-organ-sinus gland complex in the eyestalk. Additionally, this study would benefit from a mathematical model describing the changes in visual acuity that would result from the separation seen in the eye. However, this would require data on a number of variables – including curvature of the lens, refractive indices of the lens and crystalline cone, and focal distance – that have not yet been described for this species. With these limitations in mind, it is our opinion that there is a clear pattern that emerges from our results indicating that these crabs have reduced visual acuity during the molting process, which may be due to a separation between the corneal lens and the underlying photoreceptors.

The natural history of the blue crab is well documented, and evidence indicates that mating is limited to the time period surrounding female molting, with courtship behavior involving both chemical and visual cues (Van Engle, 1958; Teytaud, 1971; Gleeson, 1980). Males are described as using a repertoire of visual cues during courtship, including waving the claws, standing tall on the walking legs and rhythmically waving the swim paddles (Teytaud, 1971). The paddling maneuver may also function in directing chemical cues towards potential mates (Kamio et al., 2008). In courting blue crabs, males should be fully capable of receiving chemical and visual cues, whereas in females, reception of visual cues will depend on the molting stage of the female receiver. If a female pairs with a male 3 to 4 days prior to molting or well after molting, visual cues may factor into her mate choice. However, because females tend to resist pairing until 1 to 2 days before molting (Jivoff and Hines, 1998), when visual acuity is poor, visual cues may not be reliable when choosing a mate and females may instead depend upon chemical cues. The visual constraints of molting may select for multimodal cues in this species. Multimodal communication is fairly common (Hughes, 1996; Hölldobler, 1999), especially in the context of mating (Papke et al., 2006; Uetz and Roberts, 2002), and signals displayed over two modalities may increase the probability of reception (Cardé and Baker, 1984; Conner, 1987). In the blue crab, chemical and visual cues may function redundantly to overcome differences in receiver ability or temporary sensory limitations.

The effects of molting on visual acuity may impact numerous crustacean groups and, more generally, arthropod groups that molt. In crustaceans with compound eyes, the corneal lenses are comprised of exoskeleton, which must be shed; therefore, decreased visual acuity during molting is likely a general phenomenon. Given the diversity of crustacean species, the extent and duration of the changes in visual acuity may be quite variable amongst species. Additionally, the linking of mating and female molting is present throughout the crustaceans, particularly in decapods (Hartnoll, 1967; Christy, 1987; Asakura, 2009). Mating concurrent with female molting has been documented in ostracods [in Cypridinae (Morin and Cohen, 1991)], isopods [in Sphaeromatidae (Shuster, 1989)], amphipods [in Gammaridae (Borosky and Borosky, 1987)], shrimp [in Caridae and Penaeidae (Bauer, 1979; Boddeke et al., 1991)], American lobster [*Homerus americanus* (Atema, 1986)] and crabs [in Portunidae and Cancridae (Christy, 1987)]. Further, in our literature research, we have found a pattern indicating that visual sexual cues are nearly non-existent (or at least not documented) in groups that

have paired mating and molting. Studies on sexual communication across Crustacea are relatively sparse, and the existing body of literature has focused on chemical and mechanical cues (Ryan, 1966; Atema and Engstrom, 1971; Hughes, 1996). The few species that have well-documented visual cues, primarily stomatopods and fiddler crabs (Salmon, 1984; Christy and Salmon, 1991; Marshall et al., 1996; Detto et al., 2006; Chiou et al., 2008), do not have concurrent mating and molting. Reduced visual acuity during molting may serve as one explanation for the lack of observed visual cues in the sexual behavior of crustaceans that pair mating and molting. Additional comparative work across multiple taxa is needed to further explore this hypothesis. More generally, our findings suggest that, in addition to the well-known physiological challenges associated with molting, the visual system is also compromised. This is an area that has been previously unexplored and should be considered when addressing the effects of molting on the life history of a species.

ACKNOWLEDGEMENTS

We thank Mark Hooper for assistance in capturing and classifying crabs and Dr Manuel Leal for commenting on earlier drafts of the manuscript. J.B. was supported in part by a fellowship from the Duke Department of Biology. S.J. was supported in part by grants from the National Science Foundation (OCE-0852138) and the Office of Naval Research (N00014-09-1-1053).

REFERENCES

- Abbott, W.** (1967). Unusual climbing behavior by *Callinectes sapidus* Rathbun (Decapoda, Brachyura). *Crustaceana* **13**, 128.
- Asakura, A.** (2009). The evolution of mating systems in decapods crustaceans. In *Decapod Crustacean Phylogenetics* (ed. K. Crandall and D. Felder), pp. 121-182. Boca Raton, FL: CRC Press.
- Atema, J.** (1986). Review of sexual selection and chemical communication in the lobster *Homarus americanus*. *Can. J. Fish. Aquat. Sci.* **43**, 2283-2290.
- Atema, J. and Engstrom, D. G.** (1971). Sex pheromone in the lobster, *Homarus americanus*. *Nature* **232**, 261-263.
- Baldwin, J. L. and Johnsen, S.** (2009). The importance of color in mate choice in the male blue crab, *Callinectes sapidus*. *J. Exp. Biol.* **212**, 3762-3768.
- Bauer, R. T.** (1979). Sex attraction and recognition in the caridean shrimp *Heptacarpus paludicola* Holmes (Decapoda: Hippolytidae). *Mar. Behav. Physiol.* **6**, 157-174.
- Benjamini, Y. and Hochberg, Y.** (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Sci. B* **57**, 289-300.
- Boddeke, R., Bosschiet, J. R. and Goudswaard, P. C.** (1991). Sex change, mating, and sperm transfer in *Crangon crangon* (L.). In *Crustacean Sexual Biology* (ed. R. T. Bauer and J. W. Martin), pp. 164-182. New York: Columbia University Press.
- Borosky, B. and Borosky, R.** (1987). The reproductive behaviors of the amphipod crustacean *Gammarus palustris* (Bousfield) and some insights into the nature of their stimuli. *J. Exp. Mar. Biol. Ecol.* **107**, 131-144.
- Bruski, C. A. and Dunham, D. W.** (1987). The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. *Behaviour* **103**, 83-107.
- Bursey, C. R.** (1984). Color recognition by the blue crab, *Callinectes sapidus* Rathbun (Decapoda, Brachyura). *Crustaceana* **47**, 278-284.
- Cameron, J. N.** (1989). Postmoult calcification in the blue crab *Callinectes sapidus*: timing and mechanism. *J. Exp. Biol.* **143**, 285-304.
- Cardé, R. T. and Baker, T. C.** (1984). Sexual communication with pheromones. In *Chemical Ecology of Insects* (ed. W. J. Bell and R. T. Cardé), pp. 355-383. London: Chapman and Hall.
- Chiou, T. H., Kleinogel, S., Cronin, T. W., Caldwell, R. L., Loeffler, B., Siddiqi, A., Goldizen, A. and Marshall, N. J.** (2008). Circular polarization vision in a stomatopod crustacean. *Curr. Biol.* **18**, 429-434.
- Christy, J. H.** (1987). Competitive mating, mate choice and mate associations of brachyuran crabs. *Bull. Mar. Sci.* **47**, 177-191.
- Christy, J. H. and Salmon, M.** (1991). Comparative studies of reproductive behavior in mantis shrimps and fiddler crabs. *Amer. Zool.* **31**, 329-337.
- Conner, W. E.** (1987). Ultrasound: its role in the courtship of the arctiid moth, *Cynia tenera*. *Experientia* **43**, 1029-1031.
- Cronin, T. W.** (2006). Invertebrate vision in water. In *Invertebrate Vision* (ed. E. Warrant and D.-E. Nilsson), pp. 211-249. Cambridge: Cambridge University Press.
- Dendinger, J. E. and Alterman, A.** (1983). Mechanical properties in relation to chemical constituents of postmolt cuticle of the blue crab, *Callinectes sapidus*. *Comp. Biochem. Physiol. A* **75**, 421-424.
- Detto, T., Backwell, P. R. Y., Hemmi, J. M. and Zeil, J.** (2006). Visually mediated species and neighbour recognition in fiddler crabs (*Uca mjoebergi* and *Uca capricornis*). *Proc. R. Soc. Lond. B* **273**, 1661-1666.
- Eguchi, E. and Waterman, T. H.** (1965). Fine structure patterns in crustacean rhabdoms. In *Functional Organization of the Compound Eye* (ed. C. G. Bernhard), pp. 105-124. Oxford: Pergamon Press.
- Gleeson, R. A.** (1980). Pheromone communication in the reproductive behavior of the blue crab, *Callinectes sapidus*. *Mar. Behav. Physiol.* **7**, 119-134.
- Hartnoll, R. G.** (1967). Mating in the Brachyura. *Crustaceana* **16**, 161-181.
- Hines, A. H.** (2007). Ecology of juvenile and adult blue crabs. In *The Blue Crab Callinectes sapidus* (ed. V. S. Kennedy and L. E. Cronin), pp. 565-654. College Park, MD: Maryland Sea Grant.
- Hölldobler, B.** (1999). Multimodal signals in ant communication. *J. Comp. Physiol. A* **184**, 129-141.
- Hughes, M.** (1996). The function of concurrent signals: visual and chemical communication in snapping shrimp. *Anim. Behav.* **52**, 247-257.
- Jachowski, R. L.** (1974). Agonistic behavior of blue crab, *Callinectes sapidus* Rathbun. *Behaviour* **50**, 232-253.
- Jivoff, P. R. and Hines, A. H.** (1998). Female behavior, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. *Anim. Behav.* **55**, 589-603.
- Johnsen, S., Widder, E. A. and Mobley, C. D.** (2004). Propagation and perception of bioluminescence: factors affecting the success of counterillumination as a cryptic strategy. *Biol. Bull.* **207**, 1-16.
- Kamio, M., Reidenbach, M. A. and Derby, C. D.** (2008). To paddle or not: context dependent courtship display by male blue crabs, *Callinectes sapidus*. *J. Exp. Biol.* **211**, 1243-1248.
- Land, M. F.** (1997). Visual acuity in insects. *Ann. Rev. Entomol.* **42**, 147-177.
- Land, M. F.** (1999). Motion and Vision: why animals move their eyes. *J. Comp. Physiol. A* **185**, 341-352.
- Land, M. F. and Nilsson, D.-E.** (1990). Observations on the compound eyes of the deep-sea ostracod *Macrocypridina castanea*. *J. Exp. Biol.* **148**, 221-233.
- Land, M. F. and Nilsson, D.-E.** (2002). *Animal Eyes*. New York: Oxford University Press.
- Mangum, C. P.** (1992). Physiological aspects of molting in the blue crab *Callinectes sapidus*. *Amer. Zool.* **32**, 459-469.
- Marshall, N. J., Jones, J. P. and Cronin, T. W.** (1996). Behavioural evidence for colour vision in stomatopod crustaceans. *J. Comp. Physiol. A* **179**, 473-481.
- McCann, G. D. and MacGinitie, G. F.** (1965). Optomotor response studies of insect vision. *Proc. R. Soc. Lond. B* **163**, 369-401.
- Molenock, J.** (1975). Evolutionary aspects of communication in the courtship behavior of four species of anomuran crabs (*Petrolisthes*). *Behaviour* **53**, 1-30.
- Morin, J. G. and Cohen, A. C.** (1991). Bioluminescent displays, courtship, and reproduction in ostracodes. In *Crustacean Sexual Biology* (ed. R. T. Bauer and J. W. Martin), pp. 1-16. New York: Columbia University Press.
- Papke, R. S., Kemp, J. D. and Rutowski, R. L.** (2006). Multimodal signaling: structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae). *Anim. Behav.* **73**, 47-54.
- Reichardt, W.** (1961). *Sensory Communication*. New York: John Wiley and Sons.
- Ryan, E. P.** (1966). Pheromone: evidence in a decapod crustacean. *Science* **151**, 340-341.
- Salmon, M.** (1984). Coastal distribution, display and sound production by Florida fiddler crabs (genus *Uca*). *Anim. Behav.* **15**, 449-459.
- Shöne, H.** (1968). Agonistic and sexual display in aquatic and semi-terrestrial brachyuran crabs. *Amer. Zool.* **8**, 641-654.
- Shuster, S. M.** (1989). Female sexual receptivity associated with molting and differences in copulatory behavior among the three male morphs in *Paracerceis sculpta* (Crustacea: Isopoda). *Biol. Bull.* **177**, 331-337.
- Smith, S. G. and Chang, E. S.** (2007). Molting and growth. In *The Blue Crab Callinectes sapidus* (ed. V. S. Kennedy and L. E. Cronin), pp. 197-245. College Park, MD: Maryland Sea Grant.
- Teytaud, A. R.** (1971). The laboratory studies of sex recognition in the blue crab *Callinectes sapidus* Rathbun. *Sea Grant Tech. Bull.* **15**, 1-63.
- Uetz, G. W. and Roberts, J. A.** (2002). Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. *Brain Behav. Evol.* **59**, 222-230.
- Van Engle, W. A.** (1958). The blue crab and its fishery in Chesapeake Bay. Part 1 – reproduction, early development growth, and migration. *Commerc. Fish. Rev.* **20**, 6-17.
- Warrant, E. J. and McIntyre, P. D.** (1990). Limitations to resolution in superposition eyes. *J. Comp. Physiol. A* **167**, 785-803.

