

Morphological colour change in the American lobster (*Homarus americanus*) in response to background colour and UV light

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Abstract American lobster (*Homarus americanus*) have a thick calcified cuticle, and do not exhibit rapid colour changes characteristic of other crustaceans. Thus, the plasticity of their coloration has been largely overlooked. Colour in lobsters is determined by the amount, location, and form of the carotenoid pigment astaxanthin, and it is possible for lobsters to alter colour by changing one of these characteristics of astaxanthin deposition. Here, short-term colour variation in American lobster in response to environmental cues (background colour and ultra-violet (UV) light) was investigated in a laboratory experiment. Lobsters were reared in conditions controlling background colour (white, black) and UV light (present, absent). Digital photographic

analysis was used to determine how these conditions influenced the luminescence (light or dark) of lobster colour, as well as the ratio of red to blue hues. Of the environmental variables considered within this experiment, UV light was the predominant factor, and caused lobsters to become darker in colour. In the absence of UV light, lobsters matched background colour, and turned darker in response to the darker background. Environmental matching has practical implications both for wild lobsters as they settle to the benthic habitat, and for enhancement programmes, to grow lobsters that are best suited for local habitats.

Keywords astaxanthin; crypsis; crustacyanin; habitat matching; phenotypic variation

INTRODUCTION

The colour of crustaceans is determined through morphological mechanisms (amount and distribution of pigments and overall structure of cuticle), or by physiological change via chromatophores (Rao 1985). Morphologic colour is less plastic and slower to change than physiologically-determined coloration (Robison & Charlton 2005). Some marine rock crab (e.g., *Cancer irroratus*) juveniles exhibit a wide variety of colours which correspond with the colours that are found in their settlement habitat (Palma & Steneck 2001). The colour differences were found to be associated with body size, as they disappeared as the crabs grew larger (Palma & Steneck 2001). Shore crab (*Carcinus maenas*) juveniles also display striking colours and patterns that correlate with the mussel beds in which they live, and may help the crabs hide from predators (Todd et al. 2006). It is possible that these examples of variable coloration may be an important strategy in reducing mortality, and Palma & Steneck (2001) suggest that it is likely more widespread in crustaceans than is currently recognised.

The American lobster (*Homarus americanus*, Milne Edwards, 1837) is located in the northwestern

Atlantic Ocean from Newfoundland and Labrador in Canada to North Carolina in the United States (Lawton & Lavalli 1995). Inshore and offshore populations are observed in a wide variety of habitats that include: mud, cobble, bedrock, peat reefs, eelgrass beds, sandy depressions, and clay (Lawton & Lavalli 1995). Predation from benthic organisms is a great threat for settled postlarvae and peat reefs have been found to provide suitable protection because, it is suggested, the lobsters may blend in with the root structures (Lawton & Lavalli 1995). The predation threat is so great that early benthic lobsters are considered restricted to shelter, and filter feed in these structures as opposed to foraging in the open (Lavalli & Barshaw 1989; Lawton & Lavalli 1995).

The most common phenotype of the American lobster is a brownish-green colour, which is affected by both dietary and genetic factors (Tlusty & Hyland 2005). Colour is controlled by the red-hued carotenoid pigments that occur naturally in the lobster's diet (D'Abramo et al. 1983). Canthaxanthin and astaxanthin (AXT) are the two dominant forms, but any canthaxanthin that is consumed will be converted to astaxanthin before incorporation into the body tissue (D'Abramo et al. 1983; Tlusty 2005). As the astaxanthin is moved from the digestive system to the epidermis, it occurs in its natural red state. When the astaxanthin is then moved into the endo- and exo-cuticle, proteins bind with it altering its tertiary structuring and creating crustacyanin (Cianci et al. 2002). Subsequently, the crustacyanin is moved to the epicuticle, where multiple crustacyanin molecules bind together. During this process, the tertiary structure of the astaxanthin is again altered, resulting in a colour change from blue to yellow (Cianci et al. 2002). Thus, the final colour of a lobster is a result of the stacking of yellow over blue over red (Tlusty & Hyland 2005). Although lobster colour is also controlled genetically, the mechanism by which phenotypic colour is controlled has not yet been revealed, though it is hypothesised that the genetic control acts over the rate of AXT uptake and carotenoprotein formation (Tlusty & Hyland 2005). These controlling factors can lead to a wide and potentially variable phenotypic colour and this study investigated whether this colour variation may afford the lobster some phenotypic plasticity.

Although the primary role of astaxanthin in lobsters is coloration, this pigment has other roles. Astaxanthin is an antioxidant, and this property has been demonstrated in a number of biological membranes (Palozza & Krinsky 1992; Oshima

et al. 1993; Nakagawa et al. 1997). It can also quench singlet oxygen activity (Shimidzu et al. 1996), scavenge oxygen free radicals and prevent lipid peroxidation (Miki 1991). Because of this antioxidant activity, astaxanthin has been proposed to be protective against ultra-violet (UV) light damage. UV light can induce photooxidation mechanisms and produce reactive oxygenative species (McVean et al. 1999; Noguchi & Niki 1999) which can then damage lipids, pigments, DNA, and proteins. Astaxanthin has been demonstrated to protect against UV light stress (Tso & Lam 1996; Kobayashi & Okada 2000), although the results are equivocal (Savouré et al. 1995; Black 1998). Nevertheless, a number of commercial sun-block lotions now contain this compound (Wolk 2005).

Three experiments were conducted to assess both the role of UV light on lobster coloration and the potential for lobsters to phenotypically match their environment. The first experiment assessed the influence of UV light; the second assessed the influence of container colour; and finally, a two-way factorial experiment that simultaneously manipulated UV light and container colour was performed. Lobster colour changes to these variables were documented and analysed in each experiment using digital photographic colour analysis (Tlusty 2005).

MATERIALS AND METHODS

Lobsters used for all three experiments were hatched and reared at the New England Aquarium in Boston, Massachusetts, United States, according to methods described in Tlusty (2005). Upon reaching settlement phase at stage IV, they were moved into individual 45 mm diameter by 35 mm deep containers, and fed Economac 4 shrimp diet (Aqua fauna Bio-marine Inc, United States) 5 days per week and Cyclopeeze™ (Argent Chemical Laboratories, United States), a copepod high in astaxanthin, 2 days per week. The lobsters were raised in one-half of a fibreglass seawater tray (193 cm × 18 cm × 2 cm) that was part of a larger 1705 litre recirculation system (10% water renewal daily). The three experiments were conducted within the same seawater tray between January 2006 and September 2007. Different groups of lobsters were used for each experiment, so that no lobster was used in more than one experiment.

For the UV light experiment, 54 stage V lobsters were separated randomly and equally into three treatment groups. They were maintained individually

in 45 mm diameter by 35 mm deep containers. The rearing facility provided broad spectrum fluorescent light to all lobsters, and the experimental manipulation consisted of filtering the UV light. One group of lobsters was covered by a black opaque cover that blocked all light. A second group was covered by a clear plastic cover that blocked UV light and a proportion of the broad spectrum. A third group was left uncovered and exposed to broad spectrum light. Light intensity was measured with an USB2000 Miniature Fiber Optic Spectrometer (Ocean Optics, United States). The sensor was placed through the bottom of a lobster container, and the measurement was made with the sensor flush to the bottom facing directly upward. Digital images of all lobsters were captured on days 1, 15, 22, 29, 42, and 60.

For the background colour experiment, 20 stage V and VI lobsters were randomly divided into two groups, and placed individually into either 5.3 cm² square black containers (B), or 6.5 cm diameter round white containers (W). (Container shape was a function of availability.) Light was assessed as previously, with the addition that measurements were made with the sensor oriented vertically, and angled toward the side of the container. Digital images were captured on days 0, 14, 21, 35, 49, and 63. Although 10 lobsters were initiated within each treatment, because of mortality, only seven and eight lobsters were analysed in the B and W treatments, respectively.

The final experiment was a two-way design where one factor was UV light (two levels, i.e., covered by a filter and uncovered), and the other was container colour (two levels, i.e., black and white). Twenty-four stage VI to VIII lobsters were placed into one of four treatments with 6 lobsters per treatment. Lobsters were placed individually in either white round (6.5 cm diam.) or black round (8.2 cm diam.) containers. Half of each colour container was exposed to broad spectrum fluorescent light, whereas half was covered by a UV filter (GamProducts, Inc., United States). Digital images of all lobsters were captured on days 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63.

Photographic sampling and statistics

In the UV light experiment, each lobster was photographed on the dorsal side of one claw. In the subsequent two experiments, each lobster was photographed on the dorsal side of the last abdominal segment. The difference between these two locations was not considered to influence results as they are both dorsal locations, and are coloured similarly within lobsters (Tlusty 2005). Photographs were

taken with a Nikon Coolpix 5000 digital camera with a Nikon SL1 Macro Cool Light attached by a UR-E6 step-down ring adaptor. The camera was set to the macro and manual settings. The shutter speed and aperture were set to 1/30 and F4.5, respectively. The flash was turned off and the picture was saved as a JPEG compressed at 1/4 the original size. The camera was placed on a small tripod and held approximately 3 cm above the lobsters. The lobsters were placed on a white rectangle, and the white balance was set before the pictures were taken to control for deviations in lighting in each picture. Each photo was imported into the SPSS SigmaScan program (Systat Software, United States). The white square was measured first, and a correction factor determined for deviation from pure white. An area approximately 1 mm² on the lobster was analysed for luminescence (a black-white index), as well as the red, green, blue hues on a scale from 0 to 255. This methodology has previously been proven effective for juvenile lobsters (Tlusty 2005; Tlusty & Hyland 2005). The ratio of red and blue coloration (R/B) was calculated, and was used as an index of dispersion of astaxanthin within lobsters (Tlusty 2005). High ratios occur when all astaxanthin is free and located in the epidermis, whereas low ratios occur when the pigment is bound to protein and located in the cuticle (Tlusty & Hyland 2005).

Lobsters in all experiments were held individually in containers, and the containers for all treatments were within a single tank. Thus, each lobster was considered the experimental unit. Repeated measures were made of individual lobsters over multiple days with the lobsters randomly allocated between experimental treatments. The data for the first two experiments (UV light and background colour) were analysed as two-way repeated measures ANOVAs (Zar 1984). In the first experiment, the first factor had three levels for UV (uncovered, clear, or black cover) and in the second experiment, two levels for the container colour (black, white). The second factor was the day of photographic sampling. If data were non-parametric, the colour scores were ranked each day across all treatments, and the rankings were analysed within the repeated measures ANOVA. Paired comparisons were made with the Holm-Sidak method (Glantz 2005). The last experiment was analysed as a two-way repeated measures ANOVA with the two factors day (10 levels with one for each day of photographic sampling) and "environmental" treatment (four levels of the combined pairings of the UV light (filtered, unfiltered) and background (black, white) treatments). A statistically significant

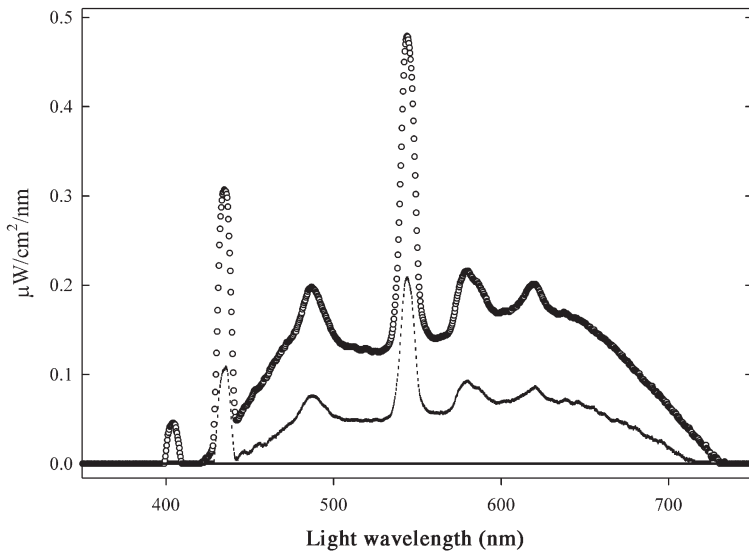


Fig. 1 Incident lighting reaching American lobster (*Homarus americanus*) directly exposed to fluorescent lighting (open symbols, top line) or covered by a clear (vertical hash marks, middle line) or black (× symbols, superimposed on 0 value of y axis) cover.

day factor would likely preclude the detection of any effect of the light/background treatments. Thus, pending a significant effect of the day factor, to detect secondary more general effects of light and background, a ranking of data by day was conducted. This analysis consisted of ranking the lobsters' colour scores across treatment each day, and then these values were assessed as a two-way ANOVA with the factors UV light and background colour (two levels per factor), with each day as an independent observation. Although this method does not consider the importance of individual lobster and the day, it was used as a second assessment of the data, after day and individual effects had been evaluated. For all tests, a significance level of $\alpha = 0.05$ was used.

RESULTS

UV Light

Measurements of the light delivered to the lobsters indicated that the open treatment received the most light, whereas the clear-covered box treatment received less light and the black box treatment received no light (Fig. 1). The open box treatment contained a UV peak at approximately 405 nm which was absent in both the clear and black covered treatments (Fig. 1).

Assessment of the lobster images indicated that the light treatment had a statistically significant effect on luminescence (two-way repeated measures

ANOVA, $F_{2,22} = 5.45$, $P < 0.01$). Neither the sample day ($F_{5,99} = 0.78$, $P > 0.5$) nor the treatment \times day interaction ($F_{10,99} = 0.50$, $P > 0.8$) were statistically different. The lobsters in the black treatment had higher average luminescence scores, and thus were lighter in colour compared with lobsters in the open treatment (Holm-Sidak test, $t = 3.30$, $P < 0.005$), but not the clear treatment ($t = 1.30$, $P > 0.2$, Fig. 2).

The ratio of red to blue pigmentation within the digital image was significantly affected by both the light treatment (ranked data, two-way repeated measures ANOVA, $F_{2,22} = 6.54$, $P < 0.005$) and the sample day ($F_{5,99} = 8.32$, $P < 0.001$), with no significant interaction of these two (ranked data, $F_{10,99} = 1.04$, $P > 0.4$) (Fig. 2). The lobsters in the open treatment had a larger ranked R/B ratio than lobsters in the other two treatments (Holm-Sidak test, $t > 2.9$, $P < 0.01$). The R/B changed with sample day, and appeared to increase toward day 29, then decreased as sampling progressed until the initial and final sample days were statistically equivalent (Fig. 2).

Background colour

The light reaching lobsters in the background colour experiment was similar in spectra to the light properties of the open treatment lobsters in the previous experiment (Fig. 3). There was slightly more total light measured in the white compared with the black containers because the light was reflected off the surfaces (Fig. 3). The colour of the

lobsters measured as luminescence did not vary with colour treatment (ranked data, $F_{1,14} = 0.60, P > 0.4$) or sample day ($F_{5,70} = 2.10, P > 0.05$). Similarly, there was no significant treatment \times day interaction ($F_{5,70} = 1.11, P > 0.3$). The R/B ratio did vary with sample day (ranked data, $F_{5,70} = 19.98, P < 0.0001$), but not treatment ($F_{1,14} = 0.96, P > 0.3$) or interaction ($F_{5,70} = 1.97, P > 0.09$). Overall, lobsters at days 49 and 63 had a lower ranked R/B ratio (mean \pm SE: $32.7 \pm 3.8, 26.7 \pm 3.1$, respectively) than on the initial days (40.3 ± 4.2).

UV light and background colour

When both background colour and the presence/absence of UV light were tested simultaneously, the lobsters experienced more total light in the white compared with the black containers (Fig. 4). The UV filter removed the peak of light at 365 nm, and approximately half of the light at 410 nm (Fig. 4). The lobster colour as measured by luminescence did not exhibit equal variance between treatments, and thus data ranked over the entire experiment were assessed. These data demonstrated a statistically significant day effect (two-way repeated measures ANOVA, $F_{9,160} = 13.26, P < 0.001$), with darker lobsters on days 42 and 63 than on the initial sampling days (for all comparisons Holm-Sidak test, $t > 3.44, P < 0.001$). The day effect was the largest effect, and neither treatment ($F_{3,19} = 1.33, P > 0.25$) nor the day \times treatment interaction ($F_{27,160} = 1.09, P > 0.35$) were significant.

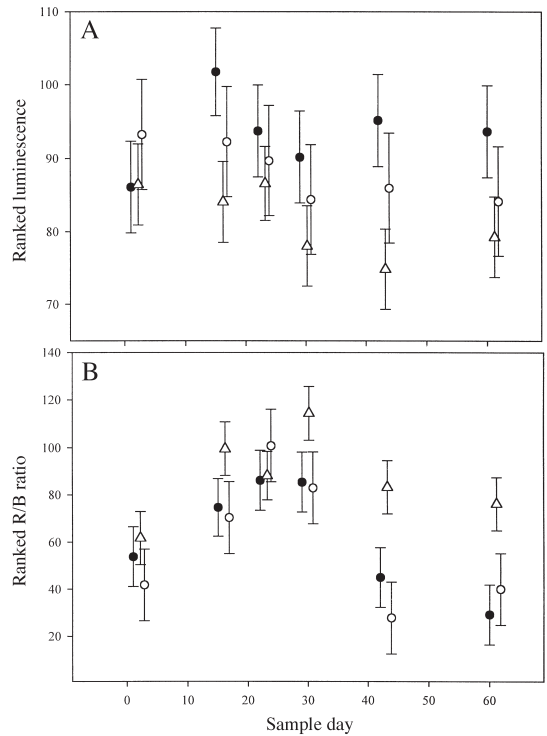
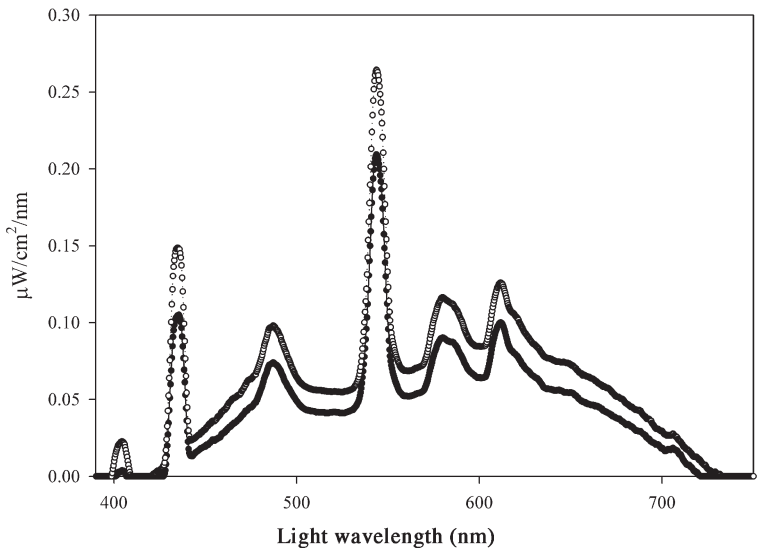


Fig. 2 A, Average (\pm 1SE) ranked luminescence; and B, R/B (red/blue coloration) ratio of lobsters ($n = 18$ per treatment) held in containers under fluorescent light without cover (open triangles), covered by a clear (open circles) or a black (closed circles) cover. A lower luminescence score indicates lobsters were a darker colour.

Fig. 3 Wavelengths of light that lobsters experienced in black (filled circles) or white (open circles) containers. Light reflecting off the sides of the white containers resulted in a slightly greater incidental light level than in the black containers.



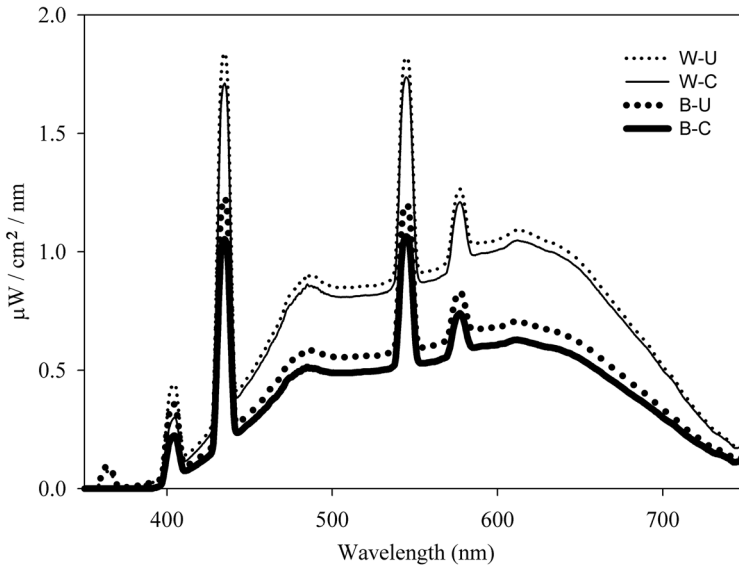


Fig. 4 Wavelengths of light that lobsters experienced in black (B) or white (W) containers that were either covered (C) or uncovered (U) by a UV light filter ($n = 6$ per treatment).

Because the day factor was significant, it may have precluded the observation of any effect of the light/background treatments. Thus, to detect secondary more general effects of light and background, the ranked data within day were analysed by a two-way ANOVA with the factors UV light and background colour (two levels per factor), with each day as an independent observation. Removing the day effect resulted in a significant light \times background interaction term ($F_{1,36} = 4.19$, $P < 0.05$). When the white and black containers were uncovered, the average luminescence ranking was similar (Holm-Sidak test, $t = 0.23$, $P > 0.8$), but when the lobster containers were covered by a UV filter, the lobsters in the black containers were significantly darker than those in the white containers ($t = 2.67$, $P < 0.01$, Fig. 4). In a similar analysis with the R/B ratio, both the container colour ($F_{1,36} = 25.68$, $P < 0.001$) and UV light treatments ($F_{1,36} = 6.74$, $P < 0.02$) were statistically significant. The R/B ratio was greater in lobsters in the white compared with those in the black containers, and in the covered compared with the uncovered treatments (Fig. 5).

DISCUSSION

Crustaceans have been shown to respond in a variety of ways to UV light. Some are photosensitive (Frank & Widder 1994) whereas others change colour (Rao 1985). UV light induces DNA damage in crustaceans, and will lead to decreased survival

in *Chasmagnathus granulata* adults (Gouveia et al. 2005). UV-A causes indirect DNA damage by the production of hydroxyl radicals which cause strand breaks and DNA cross-links whereas UV-B directly damages the DNA (Gouveia et al. 2005). In an extreme example, pigmented forms of *Gammarus pulex* survive light intensities that are fatal to the unpigmented forms (Ginet 1960).

Animals cope with UV damage in a variety of ways. In vertebrates, tanning is observed, whereas invertebrates and some poikilothermic vertebrates use physical colour changes. In the crab *C. granulata*, UV-B and UV-A radiation induce dose-dependent dispersal of pigments in the melanophores of the organism (Gouveia et al. 2004). Hansson (2004) showed that pigmentation in freshwater copepods is one way for the organisms to protect themselves from UV radiation, especially the bright red pigmentation that can be gained through astaxanthin storage. But often the concentration of carotenoids, and thus whole animal colour, depends on environmental factors, and thus the correlation between light and colour is fortuitous, and not true chromatic adaptation (Ghidalia 1985).

In our experiments on American lobster presented here, UV light alone induced a change in the overall colour intensity, a scenario analogous to the fortuitous colour change suggested by Ghidalia (1985). Lobsters exposed to UV light were darker than those held in the absence of light. Filtering the UV light also lightened the colour of lobsters, but not to the same degree as that observed in the

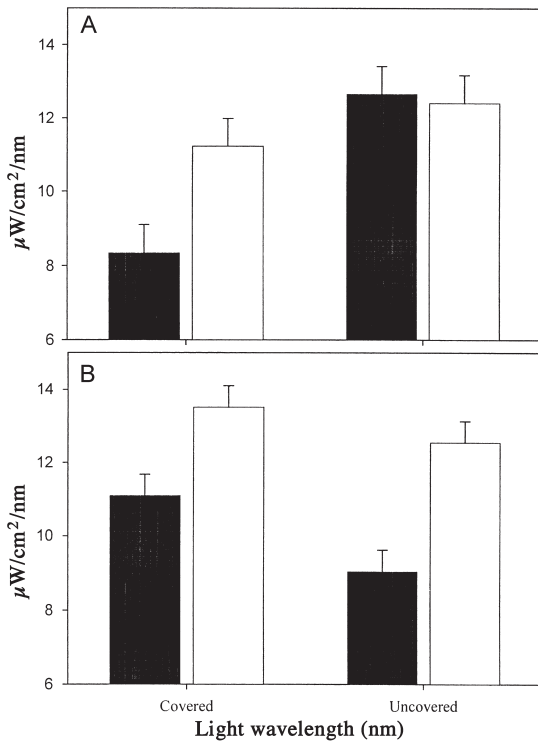


Fig. 5 A, Average (± 1 SE) rank of luminescence within day; and B, R/B (red/blue coloration) ratio of American lobster (*Homarus americanus*) held in black or white containers either covered by a UV filter, or uncovered and exposed to a broad spectrum light. For the luminescent scores, lower values indicate a darker colour.

absence of light. The results of the third experiment determined that lobsters were able to match the colour of their containers. In this example, lobsters held in a black container were darker than those held in a white container, but this occurred only when UV light was filtered and absent. In the presence of UV light, the container colour had no effect on lobster colour intensity. Thus, these data are consistent with the hypothesis that protection against UV light and subsequent protection from UV damage is a primary function of colour in American lobster. Although feasible, habitat matching is a weaker response and likely secondary. Furthermore, the overall colour shifts in American lobster observed in this series of experiments, although statistically significant, were more subtle than diet-induced colour shifts (Thlusty & Hyland 2005). Lobsters in absence of light appeared paler than those in the light treatments. This subtle colour change is consistent with colour patterns of

crustaceans with thick calcified cuticles (Ghidalia 1985) and further supports the supposition that the colour changes are not sufficient to affect crypsis, but instead are a response to UV exposure.

The ratio of red and blue coloration in lobsters has been an effective measure to examine large-scale colour changes (e.g., white to coloured, Thlusty 2005). However, it may not be as efficient on the smaller-scale changes such as differences in hue associated with background matching or in response to UV exposure. The R/B ratio is high when all astaxanthin is free and located in the epidermis, whereas the ratio is low when the pigment is bound to protein and located in the cuticle (Thlusty & Hyland 2005). In the first experiment, the R/B ratio of lobsters exposed to UV light was greater than that of lobsters not exposed to light. Yet, in the third experiment, this ratio exhibited the opposite trend. The overall rate of colour change likely varies with thickness of the component cuticular layers (and hence age of animals), as well as the overall level of pigment in the diet (Thlusty 2005). Direct testing of astaxanthin levels in the epidermis and cuticle is needed to better understand the overall use and deposition of astaxanthin in relation to UV stress.

Crustaceans as a group exhibit large differences in coloration. Those that live at depth are often uniformly red, since red wavelengths are absorbed at the surface rendering these animals invisible (Ghidalia 1985). Crustaceans living in corals have bright hues, whereas those in sand are brown, and those in seaweed are green or blue green (Ghidalia 1985). Those animals with chromatophores, or with thin translucent exoskeletons are able to mobilise pigments to effect a change in colour (Ghidalia 1985). Yet, even with their thick calcified cuticle, American lobster showed a degree of plasticity in phenotypic coloration. Although not as dramatic as a chromatophore-based colour change, the shift in hues in response to the presence or absence of UV light provides a platform to further investigate the role of pigments in responses to stressful environments.

Although crustaceans with a thick cuticle, and thus primarily dependent on morphological colour change, are less plastic in their colour variation, variation in colour still has significant survival implications. Morphological colour conditioning was used to improve the survival of hatchery-reared blue crab (*Callinectes sapidus*) released in an enhancement programme (Davis et al. 2005). The appropriate level of astaxanthin in the diet of American lobster to maintain natural colour is approximately $100 \mu\text{g}$ pigment g^{-1} diet (D'Abramo et

al. 1983). Thus, maintaining appropriate coloration for this species, and potentially manipulating it should not be a prohibitive step in an enhancement programme.

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