The role of salinity in starfish (*Asterias rubens*) colouration

Av Anke Weber and Sebastian Holmes

Dr Anke Weber is an Aquatic Ecologist at NIVA in Trondheim and *Dr Sebastian Holmes* is a Benthic Ecologist/Population Geneticist at The School of Biological Sciences in the University of Sydney, Australia.

Abstract

We tested if salinity is likely to be the main factor explaining variation in colouration of *Asterias rubens*. The common starfish (sea star) *A. rubens* is a frequent inhabitant of the Scandinavian seashores and is found in a wide variety of colours. The colour spectrum this species expresses is very broad ranging from bright purple to orange, pale brown and beige nuances. Despite displaying such a wide range of colour morphs and exhibiting the ability to alter their colouration, little is known about the mechanisms for why *A. rubens* will express particular colours morphs in particular habitats and why and how they can change their colour. In this study, the effect of two water masses on the colour morphs expressed by *A. rubens* is examined, confirming earlier field observations that colouration is related to water chemistry. It is suggested that salinity is involved shaping colouration in *A. rubens*. In saline water of 30-34 PSU the bright colours which are found in water with lower salinity (15-30 PSU) disappear.

Sammendrag

Funksjonen av saltinnhold i farging av *Asterias rubens*. *A. rubens* (Vanlig korstroll) er en kjent og vanlig organisme i mange deler av havet. Arten finnes i flere strålende farger inklusiv blank lilla, blank rosa, blank brunt og lyse lilla, lyse rosa og lyse brunt. Det er imidlertid ukjent hvorfor korstrollet har de fargene de har, og hva som styrer fargeforandringen. I artikkelen undersøker vi om fargene til *A. rubens* kan forklares av saltinnhold. Konklusjonen er at fargen til vanlig korstroll påvirkes av saltinnholdet i vannet, kanskje sammen med andre vann parametrene. I saltvann (30-34 PSU) forsvinner de blanke fargene til korstrollet (spesielt dyp lilla) som kan opptre i vann med lavere saltinnhold (15-30 PSU).

Introduction

Variation in colouration is a common phenomenon (Anger et al., 1977; Czeczuga, 1977; Stancyk & Shaffer, 1977; Russell-Hunter 1979; Arakai & Uehara, 1991, Harley et al., 2006) in a variety of echinoderms. It is often assumed that different colour morphs simply reflect phenotypic variation however, very little is known about what cause colouration and the processes leading to colour change (Harley et al., 2006). For example, the Hawaiian rock boring sea urchin, *Echinometra mathaei*, has a widespread distribution and a wide range of coloration, which has lead to the conclusion that different colour morphs may be different species (Arakai & Uehara, 1991).

Harley et al. (2006) have investigated colour variation in the Purple Ochre Sea Star (*Pisaster ochraceus*). They found that colour frequency was unrelated to the genetic structure of a population, adult size and/or to the frequency of environmental perturbation a population experienced. This led them to propose that there was some relationship between the expressed colour morph, diet and salinity. Correspondingly, Stancyk and Shaffer (1977) have found that populations of the Angular brittle star, *Ophiothrix angulata*, have different colour varieties along the west coast of North America that is linked to salinity.

The common sea star is a frequent inhabitant of the benthos in the northeast Atlantic and Indian Ocean (Anger et al., 1977, Weber et al., 2004). It is found in a wide variety of colours: including bright purple, bright orange and bright rose shades (Czeczuga, 1977). Deeper water specimens exhibited a very different colouration to shallow water conspecifics at the same location (Anger et al., 1977; Coteur et al., 2003). This has led Coteur et al., (2003) to suggest that salinity may be responsible for colour variation in *A. rubens,* although only a black morph was typically of low salinities for the sites studied.

In this study, the effect of two water masses with different salinity regimes on the colouring expressed by *A. rubens* is investigated experimentally.

Materials and methods

All experiments were carried out at The Sven Lovén Centre for Marine Sciences (**www.loven.gu.se)** formally known as the Kristineberg Marine Research Station (KMRS). All specimens were obtained from the Gullmar fjord on the Sweedish west coast. The fjord is 30 km long with a maximum depth of 118 m and has three distinct water masses: 1) surface water of varying salinity depending on the input of local runoff; 2) an intermediate water layer dominated by Skagerrak surface water; 3) Bottom water with high salinity derived from North Sea bottom.

The experiments carried out can be split into 2 parts, an adaptation period (collection, starvation and feeding) and the experimental period.

Adaptation period

Eighty individuals were collected from 1m depth (water mass 1) in the Gullmar fjord near the research station and their colouration noted. Forty randomly

selected specimens were then placed in a flow-though tank (42x21x25 cm) supplied (1.3 l min^{-1}) with surface water at 15 to 30 PSU (Lindahl, 2009). The remaining 40 specimens were placed in a second tank supplied (1.3 l min^{-1}) with deep water at 30-34 PSU, (water mass 3) from 30 m depth (Lindahl, 2009). We chose to conduct the adaptation period under experimental conditions rather than adapting all *Asterias* in water with f.ex low salinity. This was done to minimize potential adaptational effects on the experimental group that has to adjust to a new (higher) salinity level right at the start of the experiment since this extra challenge, which the other group does not have, might lead to a change in response capacity respectively to the readily adapted group Coteur et al., 2003. Both tanks were subject to ambient illumination and maintained at 11.5°C. For the first two days all specimens were starved and their colour noted at the end of the starvation period. They were then subsequently fed, each morning, for the next two days with the blue mussel, *Mytilus edulis*. All individuals were observed to feed and any colour changes were recorded. At the end of the adaptation period, the 20 individuals with the brightest purple, rose and the darkest brown colouration were selected from each treatment and measured (largest radial diameter). All individuals selected looked healthy and had undamaged arms.

Experiment

During 6 consecutive days the *A. rubens* were inspected for their colour every morning at 09.00 hours. This colour check was undertaken by 3 independent volunteers separately. They used provided colour cards for their judgement and were not informed about the sort of treatment or the theory behind the experiment.

Statistical analysis

The equality of sizes of the *Asterias* assigned to the two treatments was tested with a Student"s t-test. The homogeneity of the results of the daily colour observations by the volunteers was tested using a Mann-Whitney test. The effect of the independent variable "water type (surface/deep)" was tested by a one-way-analysis of variance (ANOVA). Significant water type effects were further explored with the Tukey HSD test (Sokal & Rholf, 1981). Prior to analysis, the data were tested for normality with the Wilk-Shaprio procedure after checking for equality of variance with Levene"s Test.

Results

Adaptation period: Initial colouration

During the initial two day starvation period, all specimens in both treatments initially lost some of the brightness of their colour. In the surface water treatment the Bright Purple organisms regained their bright colour already during the starvation period, while in the deep water treatment only some individuals regained Bright Purple. The colour distribution at the beginning of the core experiment (after the adaptation period) was 7:7:6 (Dark Brown: Bright Purple: Bright Rose) individuals in both treatments, table 1.

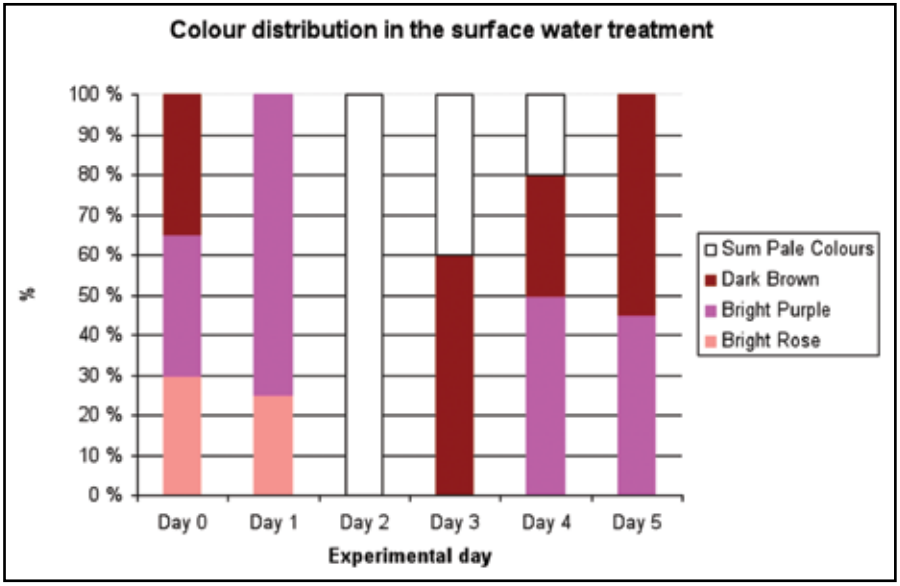
Colour changes

The means and averages of the colour changes are given in Table 1. Summarized over the whole experimental period, in the surface water mainly Bright Purple and Dark Brown (41 and 36 individuals, respectively) *Asterias* were found while in the deep water Dark Brown and Pale Purple (55 and 24 individuals, respectively) were the dominating colours, table 1.

The relative colour changes over the 6 day experimental period are given in figure 1.

Water type	Colour	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Average
surface	Pale Rose	-	-	6	-	4	-	10
surface	Pale Purple	-	-	8	8	-	-	16
surface	Pale Brown	-	-	6	-	-	-	6
surface	Bright Rose	6	5	-	-	-	-	11
surface	Bright Purple	7	15	-	-	10	9	41*
surface	Dark Brown	7	-	-	12	6	11	36
deep	Pale Rose	-	4	-	-	-	6	10
deep	Pale Purple	-	4	-	20	-	-	24
deep	Pale Brown	-	8	-	-	-	4	12
deep	Bright Rose	6	-	-	-	6	-	12
deep	Bright Purple	7	-	-	-	-	-	7*
deep	Dark Brown	7	4	20	-	14	10	55

*Table1. Results of the colour distribution per water type on consecutive days. single-factor ANOVA. * indicates a significant results at the 0.05 level.*



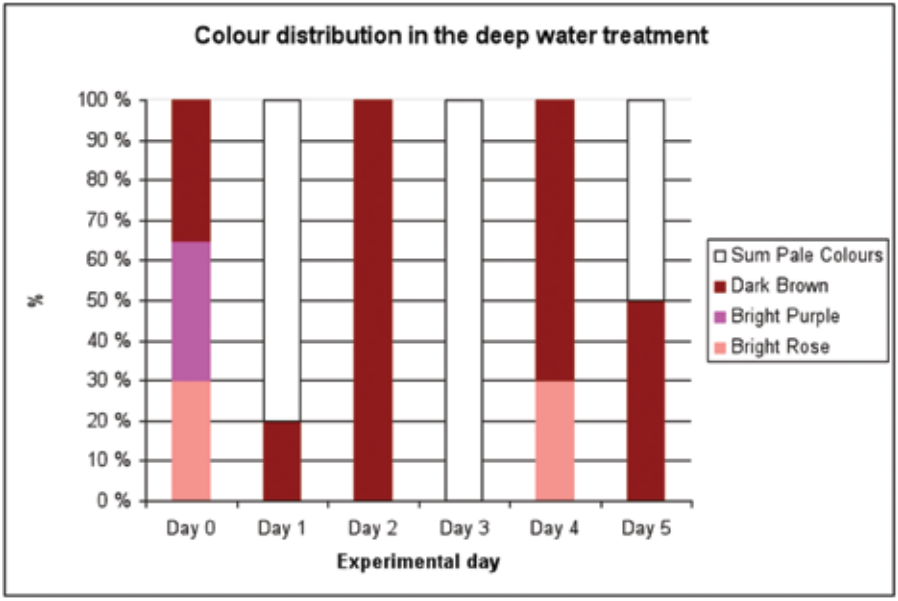


Figure 1. Observed colour change in the surface water treatment and deep water treatment over 6 days. Since no significant differences were found for the three pale colours, they have been merged in this figure for clarity. At the start of the experiment the distribution of colours was equal in both treatments. In the course of 6 days the Bright Purple colour disappeared from the deep water treatment but not from the surface water treatment (p< 0.04).*

Statistical analysis

Analysis of the sizes (largest radial diameter) between treatments showed no statistically significant difference (Students T-test: p= 0,49). The mean diameter ± standard deviation recorded was 10 ± 2 cm. Comparison between the observers in terms of daily colour matching revealed no statistically significant difference (p= 0,78) i.e. there was no difference between observers in there allocation of a starfish to particular colour category.

The results from the ANOVA are presented in table 2.

These results show a significant difference ($p<0.05^*$) was found between the two water types. The Tukey HSD test revealed that the only significant colour difference between surface water and deep water was found for Bright Purple ($p<0.05^*$), which was present more frequently and more consistently in the surface water treatment, table 1 and figure 1.

Water type	Colour	Df	df error	MS error	MS effect
Surface	Pale Rose	6	10	1,667	7,07
Surface	Pale purple	6	16	2,667	17,07
Surface	Pale Brown	6	6	1,000	6,00
Surface	Bright Rose	6	11	1,833	8,17
Surface	Bright Purple	6	41	6,833	34,97*
Surface	Dark Brown	6	36	6,000	26,80
Deep	Pale Rose	6	10	1,667	7,07
Deep	Pale purple	6	24	4,000	64,00
Deep	Pale Brown	6	12	2,000	11,20
Deep	Bright Rose	6	12	2,000	9,60
Deep	Bright Purple	6	7	1,167	8,17*
Deep	Dark Brown	6	55	9,167	51,37
Source of Variation	**SS**	**df**	**MS**	**F**	**p-value**
Between Groups	454,667	11	41,333	1,972	0,047*
Within Groups	1257,333	60	20,956		
Total	1712	71			

*Table 2. Results of the single- factor ANOVA. * indicates a significant results at the 0.05 level.*

Conclusions

Our results confirm the hypothesis that coloration appears to be, at least in part, genetically independent (Harley et al., 2006) since all our experimental animals were caught in one sampling session at the same location and were randomly assigned to either treatment. It appears that although the range of possible colours may well be genetically determined, all individuals can adopt at least one pale and one bright colour (Table 1: surface water: bright colours on Day 0, pale colours on Day 2; deep water: bright colours on Day 0, pale colours on Day 3). In addition, temperature as determining factor for the differences in the colour change between the two treatments can be ruled out since both treatments were run at 11.5°C. Neither does coloration seem to be linked to diet, a hypothesis of Harley et al. (2006), since the experimental animals received the same food prior to the beginning of the experiment, but to be absolute certain, this aspect would have to be further explored. A last hypothesis (Anger et al., 1977; Coteur et al., 2003; Harley et al. 2006) states that coloration is governed by water chemistry. Our results show that there is a significant difference in the frequency of the colour Bright Purple between the surface and the deep water treatments. This is in line with the findings that "deeper water specimens exhibited a very different colouration to shallow water con-specifics at the same location" (Anger et al., 1977; Coteur et al., 2003). Although the colour frequencies changed markedly from day

to day in an experimental period that might be perceived as relatively short, the difference between the treatments in the frequency of Bright Purple was significant. Bright Purple disappeared from the deep water/high-salinity treatment after Day 0, figure 1. This shows for the first time experimentally that there is a clear trend towards a situation in which Bright Purple starfish is present in the surface water treatment and absent in the deep water treatment, and confirm earlier speculations about salinity playing a role in sea star coloration (Anger et al., 1977; Coteur et al., 2003; Harley et al. 2006).

Therefore, we conclude that the change in coloration, at least the loss of the colour Bright Purple in the deep water treatment is triggered by higher salinity, relative to the surface water. This result is in concert with the findings for *P. ochraceus* (Harley et al., 2006) who belongs to the same family as *A. rubens* and strengthens the hypothesis that polychromatism in Asteriidea is governed predominantly by salinity.

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