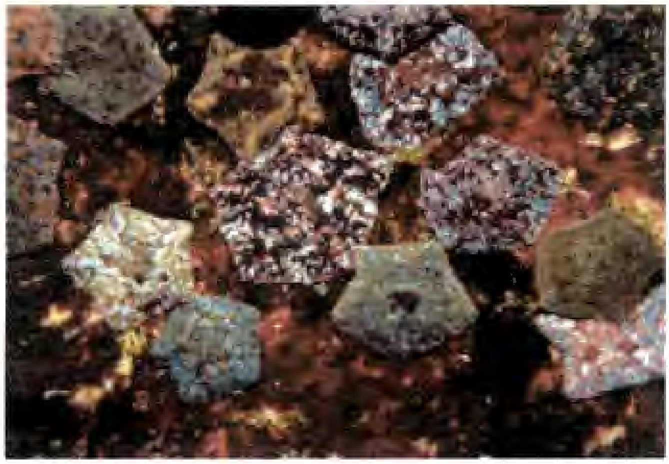
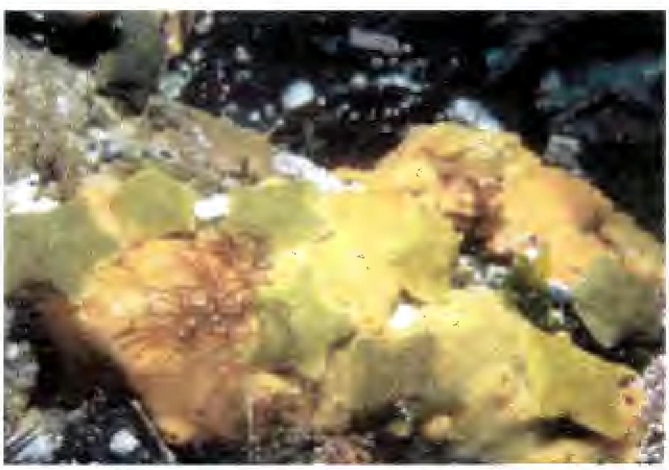
Life on a gradient: Activity levels of the seastar *Patiriella exigua* in different abiotic conditions





Top: The mottled morph of *Patiriella exigua*, found on the east coast of South Africa
Bottom: The green Morph of *Patiriella exigua*, found on the west coast of South Africa (Photographs by C. Griffiths)

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Abstract

Two morphs of *Patiriella exigua* occur in South Africa. These morphs occur on opposite sides of a temperature divide present at Cape Point. The green morph occurring on the West Coast (cold temperate) and the mottled morph occurring on the South Coast (warm temperate). In a previous study by Katherine Dunbar these two morphs were shown to be the same species. This project tests if there is a physiological difference between the two morphs of *P. exigua*. This was achieved by observing the activity coefficient of righting response of the two morphs of *P. exigua* under different temperatures and dissolved oxygen concentrations. The green morph was collected from Green Point (West Coast) and the mottled morph from Kalk Bay (South Coast). The results of a general linear model indicated that temperature and dissolved oxygen were significant factors determining righting time of *P. exigua* with P-values of less than 0.05. A T-test indicated that there was a significant difference between the righting times of the two morphs of *P. exigua* with the green morph turning at a faster rate than the mottled morph when placed under similar conditions.

1. Introduction

1.1. Background

Patiriella exigua is a small sea star (Echinodermata: Asterinidae) with a worldwide distribution ranging from the Atlantic Ocean to the Pacific Ocean (Dartnall, 1971; Clark and Downey, 1992, Fig.1) and has been recorded along the African coast from southern Namibia to southern Mozambique. The species has also been recorded from two oceanic islands in the southern Indian Ocean: Amsterdam and St. Paul Islands. *P. exigua* is absent from Western Australia, but is widespread in the southeast and around Lord Howe Island (Waters and Roy 2004). The species was first described nearly 200 years ago (Lamarck, 1816; type locality unknown), and South African specimens were collected as early as 1829 (Waters and Roy 2004; see Fig.1.)

P. exigua has an entirely benthic life history and is restricted to rock pools and shallow subtidal waters (maximum depth of 3 m), where it lives on and under stones. *P. exigua* produces egg masses that are entirely benthic and develop without parental care (Byrne 1995; Byrne *et al.*, 1999). It is largely a micophagous scavenger, extruding its stomach onto the surface of rocks to feed on diatoms, spores, detritus and presumably bacteria (Branch and Branch 1980). *P. exigua* were also noted with there stomach extruded on macroalgae and on dead animals (Pers. obs.).

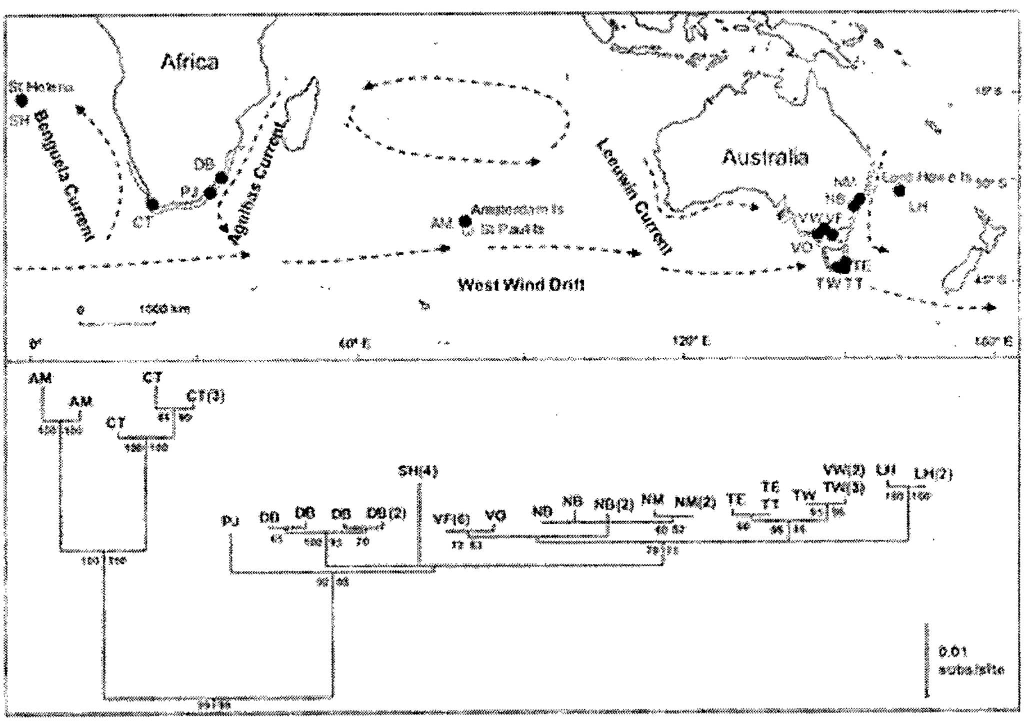


Figure 1: Geographic distribution of *Patiriella exigua* in the Southern Hemisphere, with known records indicated by dotted lines. Dashed lines represent major ocean currents. Phylogenetic relationships of *P. exigua* haloptypes (In likelihood = -5007.70) are indicated by location code below the map. (Waters and Roy 2004)

1.2. Hypotheses

This honours project is linked to a Ph.D Project by Katherine Dunbar, who has performed DNA analyzes on *P. exigua* found along the South African Coast and has found the two morphs of *P. exigua* (mottled and green) in South Africa to be the same species. Her project was funded by the UK Natural Environment Research Council, which is using *Patiriella* as a model to explore the evolutionary mechanisms responsible for phenotypic divergences and speciation along an intertidal abiotic gradient.

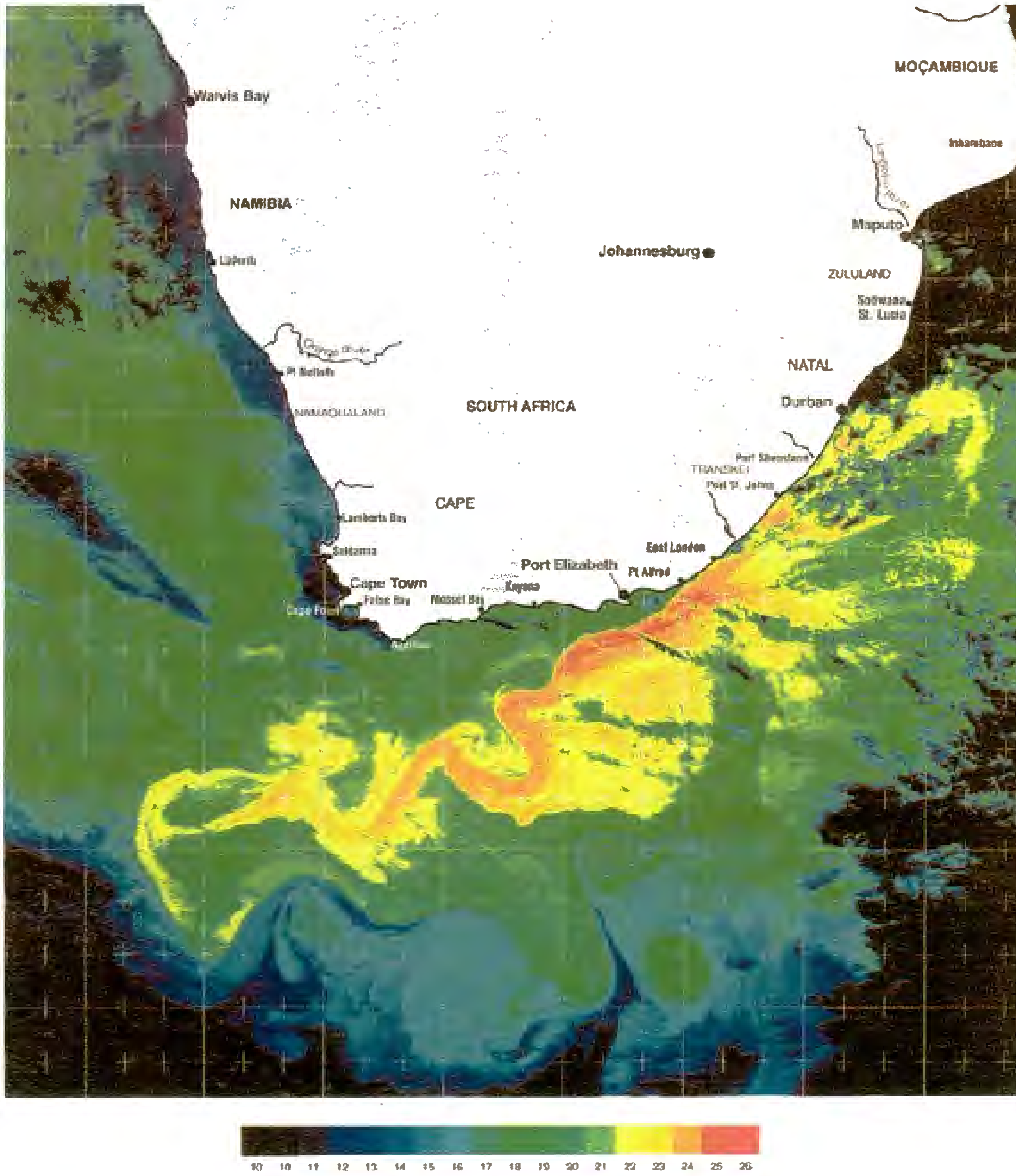
This project looks to see whether there is any physiological difference between the two morphs of *P. exigua.* The main questions to be answered were:

1. Are green animals more tolerant to fluctuations in temperature than mottled animals?
2. Are mottled animals more tolerant to fluctuations in dissolved oxygen (DO) than green animals?

These hypotheses were tested by observing the righting response of *P. exigua* under different temperature and dissolved oxygen concentrations. Also, by ascertaining which abiotic factors *P. exigua* are sensitive to, the results of this experiment may give clues as to what selection pressures *P. exigua* are facing and what adaptations they have evolved to survive in such a wide variety of environmental. It has been shown that out of salinity, pH, carbon dioxide, dissolved oxygen and temperature the latter two vary the most in the rock pool environment (Huggett and Griffiths, 1986).

The South African coastline can be divided into three coastal regions: West Coast (cold temperate), South Coast (warm temperate) and East Coast (subtropical). The South Coast is considered to be a transition zone between the cold temperate West Coast and the warm subtropical East Coast regions (Stegenga and Bolton, 2002,). In this area the Benguela is fed by the cold South Atlantic current and by the leakage of warm water from the Indian Ocean or Agulhus Current (Shannon, 2001, see Fig. 2). The West Coast along which the Benguela flows is characterized by coastal upwelling – the process whereby cold subsurface water is brought to the surface near the coast as a consequence of longshore equatorward winds (Shannon, 2001). Coastal upwelling occurs when long shore winds displace warm surface water equatorwards and offshore (as a consequence of the earth's rotation); resulting in a drop in sea-level against the coast and an uplift of water from below (and alongshore) to correct the imbalance caused (Shannon, 2001).

The cold Benguela current is unique among eastern-boundary upwelling systems in that it is bordered at both the northern and southern ends by warm water systems viz. the Angola and the Agulhas currents respectively. It is comprised of two major ecosystems (the southern and northern Benguela systems) separated by the principal upwelling center in the vicinity of Luderitz (27 °S:15 °E) where upwelling-favorable winds blow throughout the year (Shannon and Pillar, 1986; cited in Heymans *et al.* 2004).



Satellite photograph of the coast of Southern Africa

The mighty Agulhas Current drives down the East Coast of southern Africa, bringing warm water (red) from the tropics. On the West Coast, cold, nutrient-rich upwelled water (blue-black) drifts northwards. Five biogeographic provinces can be recognised. The cold temperate Namib Province runs from northern Namibia to Lüderitz; the cold temperate Namaqua Province from there to Cape Point; the warm temperate Agulhas Province from Cape Point to northern Transkei; the subtropical Natal Province from there to southern Moçambique; central Moçambique is tropical, but the precise boundaries of this province have not been defined.

Figure 1: Satellite photography of the coast of Southern Africa showing differences in temperature between the currents and highlighting the upwelling region along the West Coast (from Branch *et al.* 1994). This map show the temperature divide, present at Cape Point, between the West coast and South coast

2. Methods

2.1. Study Sites

Samples were collected from two study sites, one along the West Coast and the other along the South Coast. Individuals of the green phenotype were collected at Green Point, Table Bay, approximately 50 km from Cape Point. Individuals of the mottled phenotype were collected in the Muizenberg / Kalk Bay region along the South Coast, approximately 28 km from Cape Point (Fig. 3).

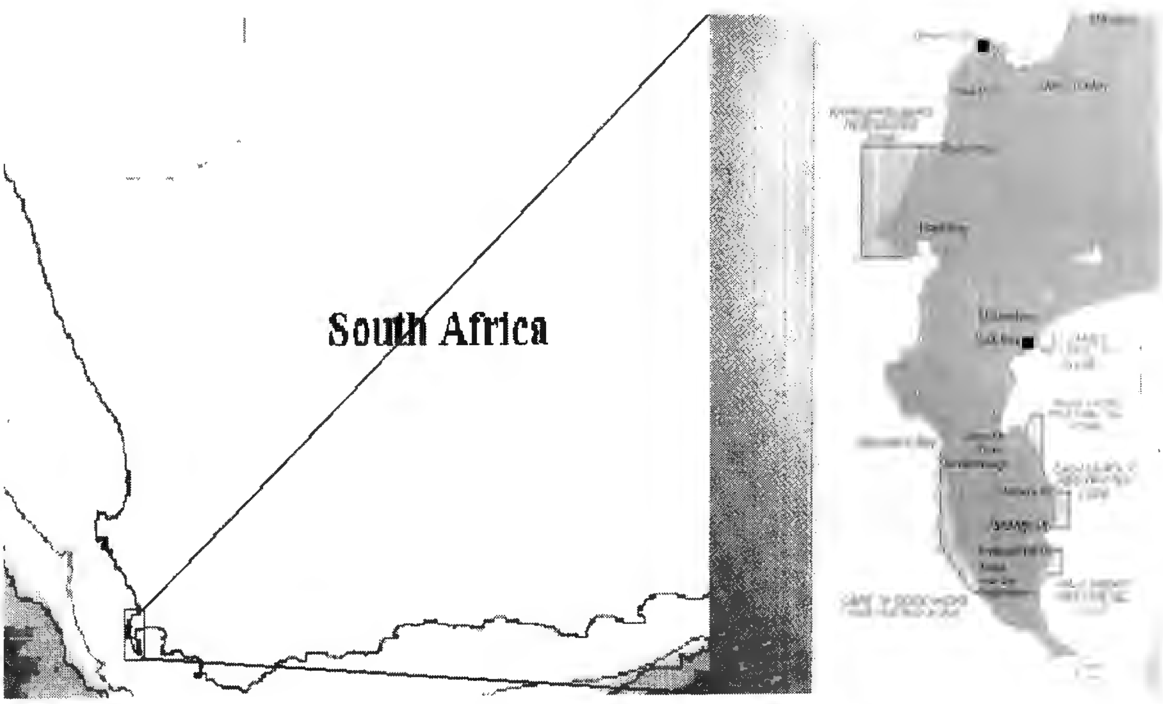


Figure 3: Study sites where samples were collected. Dark blocks (■) indicate study sites which are located in Green Point and Muizenberg / Kalk Bay, Cape Town, South Africa.

2.2. Analyses performed

Starfish were collected on several occasions between 23/05/05 – 11/06/05 at either the Green Point or Muizenberg sites. Individuals greater than 2 cm were randomly chosen from a single rock pool, the temperature of which was recorded to the nearest degree using a mercury thermometer. Collected individuals were transported to the laboratory in a container of seawater collected from the rock pool in which they were found and then placed in rectangular containers with dimensions 14.5x9.5x5 cm, with a maximum water containing capacity of 650 ml. These were placed in water baths to reach the desired temperature ranging from 10-30 °C (using increments of 5 °C). The bottom of the containers were lightly sanded with fine grain sanding paper (Silicon Carbide 220) to roughen the surface because a preliminary study showed that individuals could not turn themselves over, if the surfaces of the containers were too smooth.

Containers containing five starfish each were placed in water baths in a constant temperature room at the desired temperature and left to acclimatize. The DO ? concentrations of the containers were then manipulated to obtain the desired concentrations by bubbling Nitrogen through the water to lower the DO level or Oxygen to increase the DO level. DO was not measured at the sites due to the high variability of DO. Each starfish was then allowed to right itself three times and the average of these three turns was taken to be one reading. DO was measured using a YSI Dissolved Oxygen meter. Salinity was measured using a YSI Conductivity meter.

Statistical analyses were performed using Statistica 7. A General Linear Model (GLM) was performed to see which variables contribute to righting time (i.e. DO, Temperature and/or Form/Morph). A T-test was performed to see if there is a significant difference between righting times of the two morphs of *P. exigua*. Graphs for temperature against righting time and dissolved oxygen against righting time were also constructed.

3. Results

The dates of collection for the different morphs of *P. exigua*, and the natural conditions at the collection sites are listed in Table 1. Water temperatures ranged from 11-17 ˚C in Kalk Bay compared to 14-17 ˚C in Green Point. Salinity ranged from 31.3 ppt to 32.2 ppt. Dissolved oxygen was also recorded to get an estimation of the natural dissolved oxygen found in the rock pools from which the samples were collected and ranged from 4.2 – 8.9 ppm.

Table 1: Dates of collection for the different morphs of *P. exigua* as well as the natural conditions, which include weather (raining or not), water temperature (to the nearest degree), salinity (ppt) and DO (ppm).

Date	Morph	Raining	Water Temp	Salinity	DO
03/06/2005	Green	YES	17	31.5	4.20
05/06/2005	Green	YES	14	31.3	4.40
06/06/2005	Green	NO	14	32.0	4.47
09/06/2005	Green	YES	16	30.9	7.10
11/06/2005	Green	NO	17	32.2	8.03
23/05/2005	Mottled	NO	17	31.0	8.90
04/06/2005	Mottled	YES	15	31.9	4.80
07/06/2005	Mottled	NO	16	32.1	6.14
08/06/2005	Mottled	YES	11	31.4	6.52
10/06/2005	Mottled	NO	16	32.2	6.70

I thought the DO was not measured at site.

When righting time (in seconds) was measured against temperature it was observed that righting decreased as temperature increased. This negative relationship is highly correlated with a p-value of less than 0.05 (Figure 4). There was also a negative relationship between DO and righting time, but this relationship was not significant with a P-value of 0.5544 (Figure 5). From Figures 4 and 5 it appears that temperature is the major factor determining righting time of *P. exigua*.

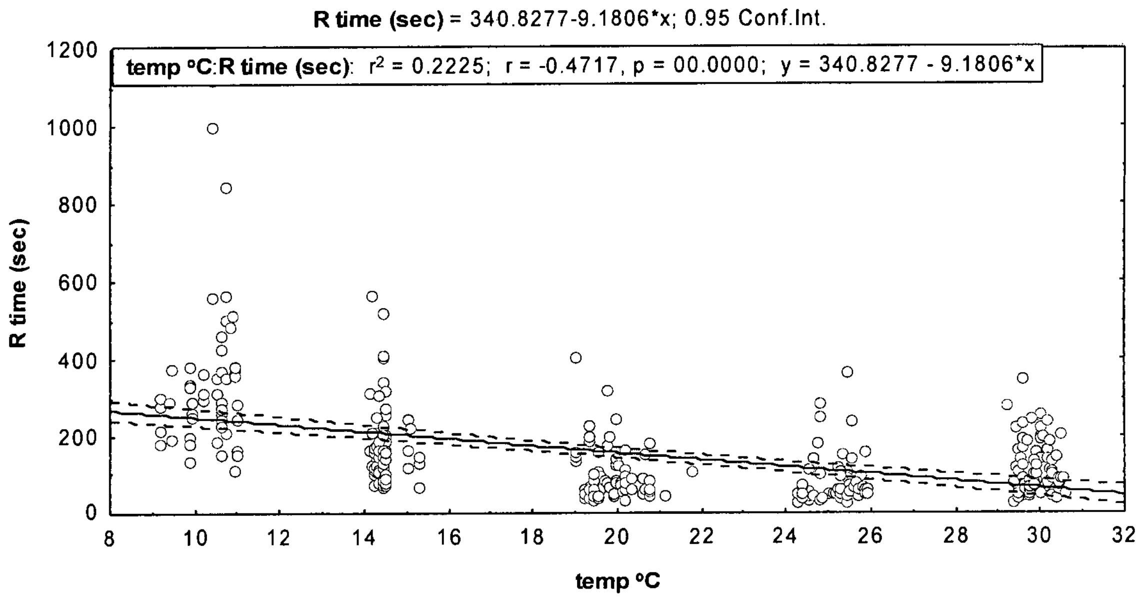


Figure 4: Shows Temperature °C Vs Righting time in seconds for both Mottled and Green form From this graph it can be observed that temperature and righting time have a negative relationship, as temperature increases righting time decreases. The graph has an r2 value of 0.2225 and a P-value of less than 0.05.

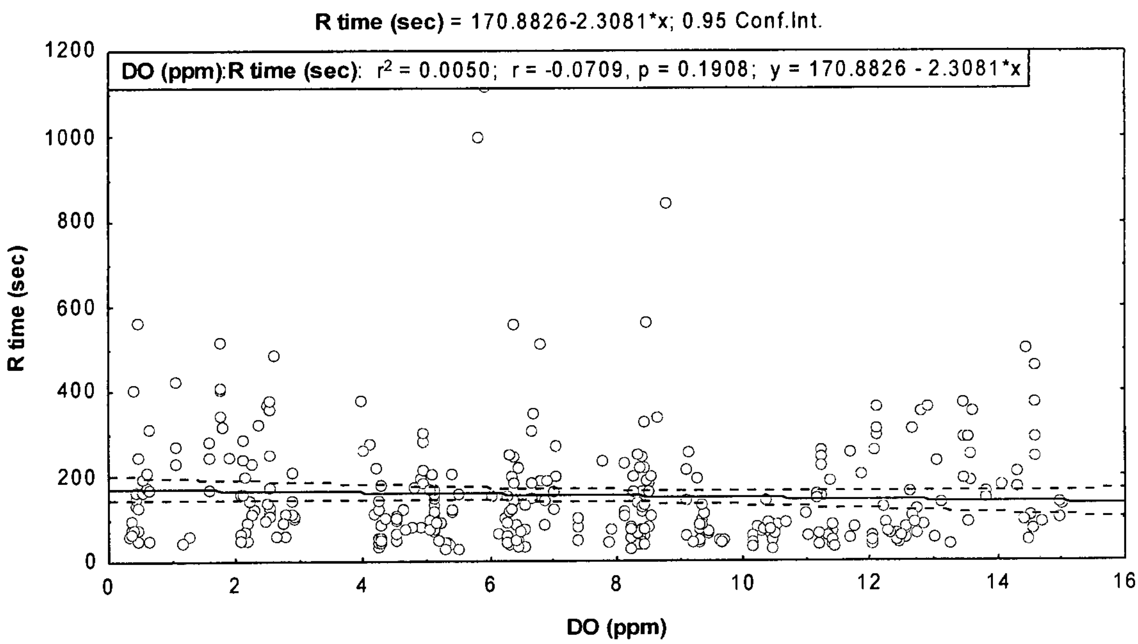


Figure 5: Dissolved Oxygen (ppm) Vs Righting time for both the mottled and the green form. The graph shows a negative relationship between dissolved oxygen and righting time. The Graph has an r^2 value of 0.0050 and a p-value of 0.1908

Figures 6 and 7 show the temperature against righting time for the mottled and green morphs of starfish and from these figures it can be observed that the average turn-over time for the mottled morph was greater than that of the green morph, which was less than 200 seconds. The turn-over times for the green and mottled morphs were both highly significant (i.e. as temperature increased righting time decreased) with P-values of less than 0.05. This shows than the green morph has a faster turn-over time when compared to the mottled morph with respect to temperature.

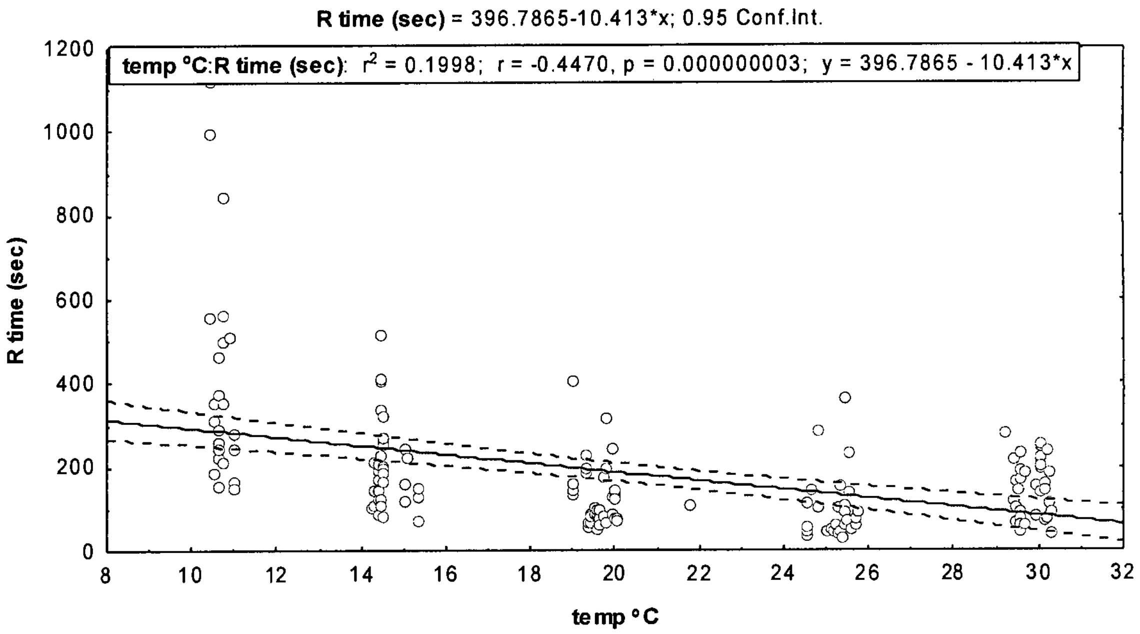


Figure 6: Righting time Vs Temperature °C for the mottled form. The graph shows a negative relationship between these two variables. The graph has an r^2 value of 0.1998 and a P-value of less than 0.05.

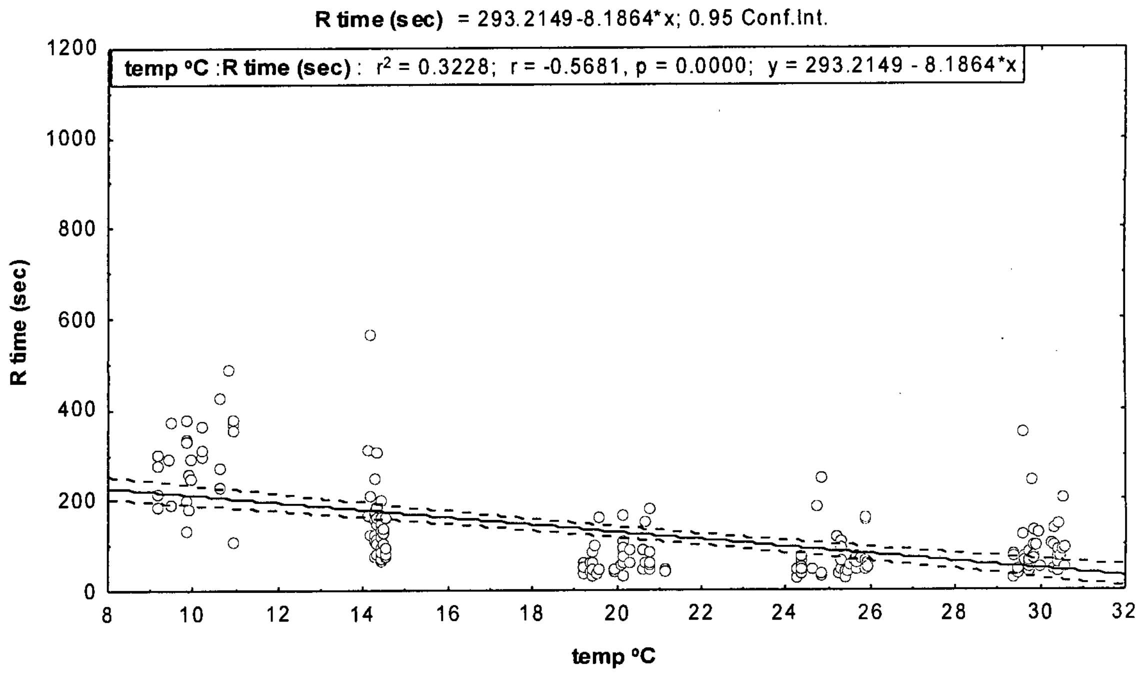


Figure 7: Righting time Vs Temperature °C for the Green form. The graph shows a negative relationship between these two variables. The graph has an r^2 value of 0.3228 and a P-value of less than 0.05

There were negative relationships between righting time and dissolved oxygen for both the mottled and green forms, but the relationship was not as significant for the mottled form with a P-value of 0.5544 as it was for the green form with a P-value of 0.0467 (Figures 8 and 9).

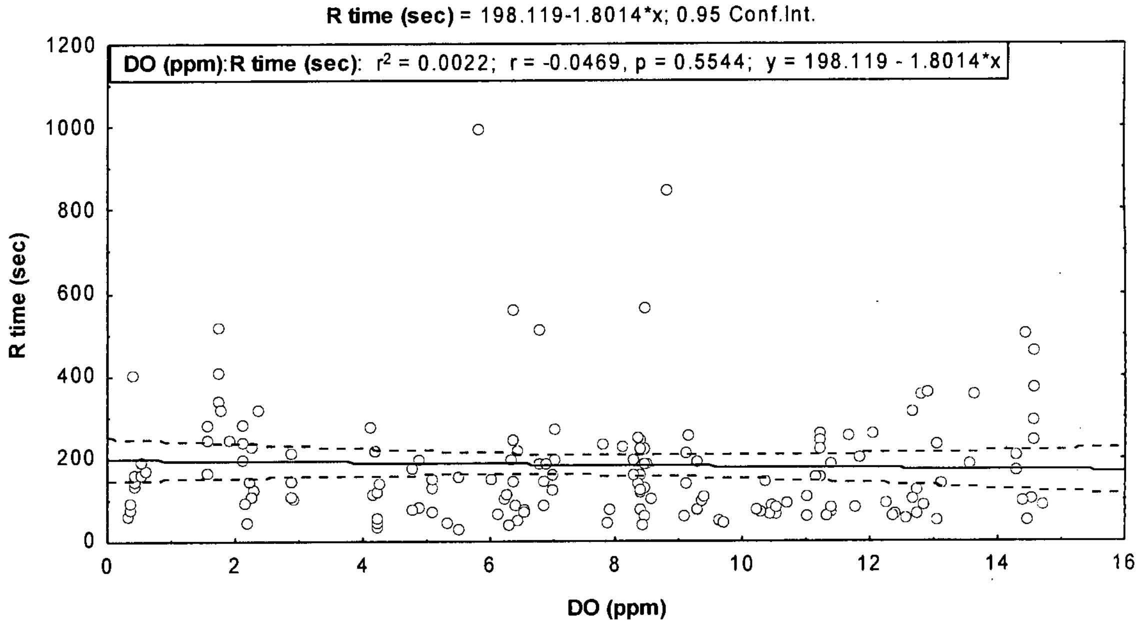


Figure 8: Dissolved Oxygen Vs Righting time for Mottled Form. The graph shows a negative relationship between these two variables. The graph has an r2 value of 0.0022 and a P-value of more than 0.05.

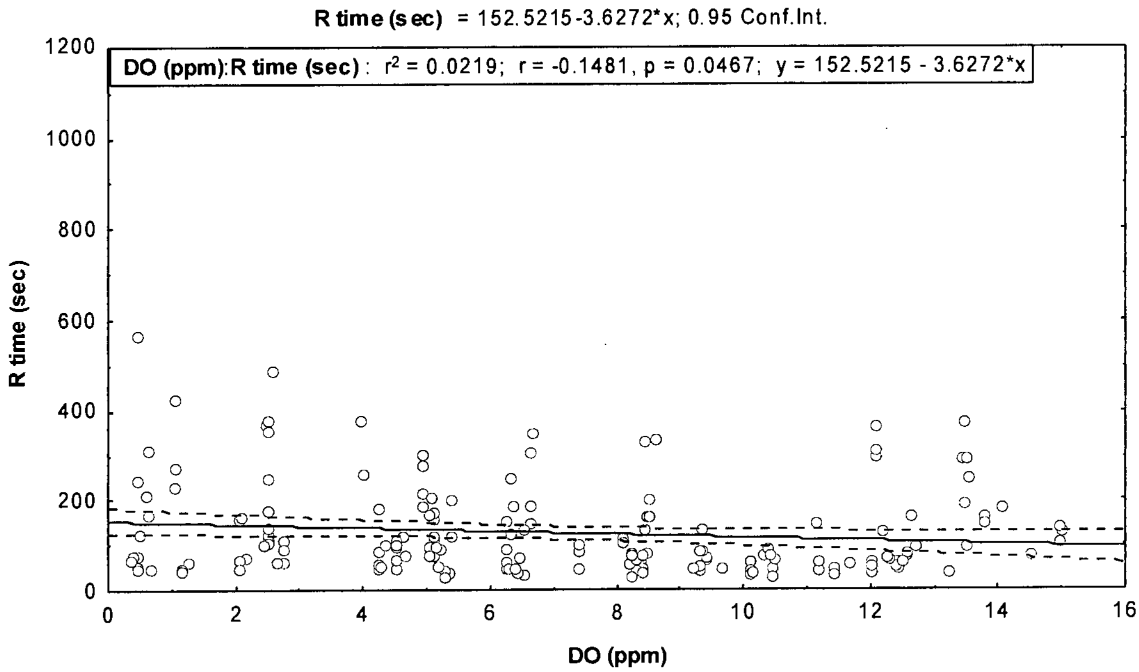


Figure 9: Dissolved Oxygen Vs Righting time for Green Form. The graph shows a negative relationship between these two variables. The graph has an r2 value of 0.0219 and a P-value of less than 0.05

Table 2 is a summary of the results of a T-test which was performed to determine whether there was a significant difference between righting times of the two morphs. The T-test showed that there was a significant difference between the righting times of the two morphs with a P-value of less than 0.05.

Table 2: Summary of T-test results between the two forms of Starfish. The T-test shows that there is a significant difference between the righting times of the two morphs. With a P-value of less than 0.05 and an F-ratio of 1.005

	T-tests; Grouping: Form (Log Raw data seconds) Group 1: Mottled Group 2: Green										
Variable	Mean Mottled	Mean Green	t-value	df	p	Valid N Mottled	Valid N Green	Std.Dev. Mottled	Std.Dev. Green	F-ratio Variances	p Variances
LOG R time	4.966657	4.594143	4.984014	340	0.000001	161	181	0.690816	0.689133	1.004892	0.972296

The righting times of the two morphs of *P. exigua* were also presented as a Box and Whisker plot (Figure 10) that and show graphically that the green morph, on average, turned faster than the mottled morph.

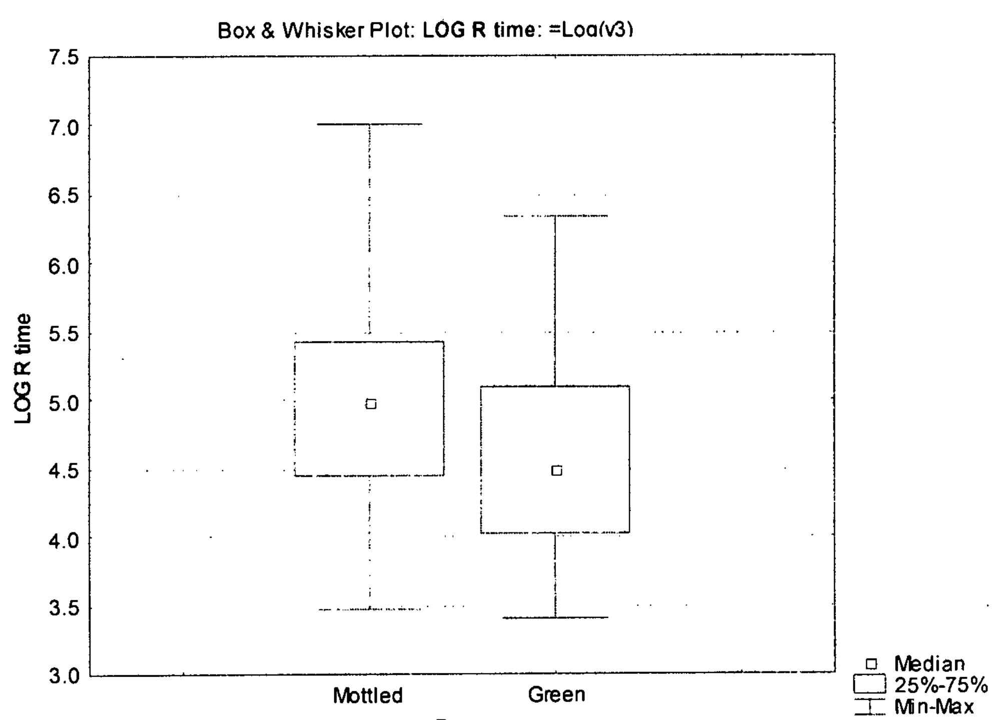


Figure 10: Box and Whisker Plot of Log Righting time Vs Form/Morph. The graph shows significant difference in turning times between the morphs.

The results of a GLM, which includes all the factors which contribute to righting time, are summarized in Table 3. It shows that dissolved oxygen, temperature and morph/form play a significant role in righting time, all having significant p-values (less than 0.05).

Table 3: Summary of General Linear Model results. Showing that dissolved oxygen, temperature and morph play a significant role in tuning time.

Effect	Univariate Tests of Significance for LOG R time Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	901.8580	1	901.8580	2673.778	0.000000
DO (ppm)	3.2240	1	3.2240	9.558	0.002156
temp C	46.1259	1	46.1259	136.751	0.000000
Form	12.8699	1	12.8699	38.156	0.000000
Error	114.0065	338	0.3373		

4. Discussion

The seastar *Patiriella exigua* can be found all along the coast of South Africa in intertidal rock pools. Although these rock pools are protected from desiccation, biota living there still have to face extremities such as alternate flooding and isolation of pools, which in turn results in varying conditions in temperature and dissolved oxygen. These cause organisms to undergo more stress than they would if they were to be exposed for longer periods of time (Newell, 1979; Huggett and Griffiths,1986).

The dates of collection for the different morphs of *P. exigua*, as well as the natural conditions were recorded in Table 1. The range in temperature indicated in Table 1 shows that there is more temperature variation in Kalk Bay area. This is due to weather conditions during the collecting from the Kalk Bay area (On the 08/06/2005, there was a cold front that past over the Cape region, which decreased the temperature). The salinity indicates the salinity ranged from 31.3 - 32.2 ppt. This is unusually low for salinity of seawater, which usually has a salinity of around 35 ppt. Rain water would drop the salinity present in the pools, but not by such a big factor as shown the range which I recorded. This leads to the conclusion that the salinity meter was probably not calibrated properly, resulting in underestimation of the salinity.

Temperature of a rock pool can be altered by various environmental conditions such as the duration of exposure and rate of heat input the pool receives. Temperature is also determined by the height of the pool in relation to tidal range, as well as air temperature, the intensity of the solar radiation received and to a lesser extent humidity and wind speed (Huggett and Griffiths, 1986).

The green morph occurs higher up the shore than the mottled morph (pers. comm. C. Griffiths, Zoology Dept., University of Cape Town). As a result the green morph would be exposed to higher fluctuations in temperature. The green morph also only occurs on the ~~on the~~ West coast of Cape Town, which is cooler due to upwelling (Figure 2), a condition that contributes to the temperature fluctuations which the green morph experiences.

The mottled morph of *P. exigua* was noted to occur more on the mid shore, where the temperature fluctuation should not be as great as those that the green morph experience due to the difference in height (in relation to tidal range). The mottled morph was collected on the South coast of the Cape Peninsula where the water is warmer due to it receiving its water from the Indian Ocean via the Agulhus Current. Thus the mottled morph should not experience the same fluctuations in temperature as the green morph. The major difference between the two sites would thus be temperature with the warmer

Indian Ocean on the South coast (warm temperate) and the cooler Atlantic Ocean on West coast (cold temperate), with the divide at Cape Point.

When righting time (in seconds) was measured against temperature it was observed that temperature and righting time had a negative relationship (i.e. as temperature increases righting time decreases). This relationship is highly correlated with a p-value of less than 0.05 (Figure 4).

Figures 6 and 7 show the temperature against righting time for the mottled and green morphs and show that the average turn-over time for the mottled morph was greater than 200 seconds, whereas the righting time of the green morph was less than 200 seconds. The turn-over times for the green and mottled morphs were both highly significant with P-values of less than 0.05. This shows than the green morph has a faster turn-over time when compared to the mottled morph with respect to temperature.

The dissolved oxygen concentration within a rock pool is determined by the number of organisms within a rock pool and their metabolic activities (Pyefinch, 1943; Daniel and Boyden, 1975; Morris and Taylor, 1983). Similar to temperature, dissolved oxygen is also affected by rate of heat input, height of pool in relation to tidal range, air temperature, humidity and wind speed, except that oxygen levels declined when the pools were isolated at night when biota were more metabolically active (Huggett and Griffiths, 1986). Also, pools high on the shore supported a lower biomass, and therefore experienced only minor diurnal variations in oxygen concentration, whereas biomass on the lower shore increased dramatically, resulting in greater extremes in oxygen concentrations even though isolation periods might have been shorter (Huggett and Griffiths, 1986).

When looking at the break down of dissolved oxygen and righting time for the green morph and the mottled morph separately (Figures 8 and 9), there were negative relationships between righting time and dissolved oxygen for both forms, but the relationship between righting time and dissolved oxygen for the green form was found to be more significant with a P-value of 0.0467 (P-value of 0.5544 of mottled form). This was unexpected; I would have assumed the mottled morph to show more of a correlation to dissolved oxygen, due to its presence in the mid-shore, where more biota should be present in the pools. Both the green morph and the mottled morph were collected from the mid-shore for this experiment; the presence of more biota in pools in the Green Point area may be a possible answer for this (percentage of the biota present in the pools should be recorded for future studies).

Possible future studies

In the future it would be useful to record air temperature when recording the water temperature in the pools, so that one could see if there is a link between water and air temperature. It would also be useful to record the percentage biomass (per species) present in the pools where the starfish are collected, this could be used to link the dissolved oxygen ranges which the starfish experience. A relocation experiment could be conducted, moving the mottled morph from Kalk Bay to Green Point (and vise versa); to see whether starfish are able to survive in these different environments.

5. Conclusions

The T-test showed that there was a significant difference between the righting times of the two morphs with a P-value of less than 0.05, which indicates that there is a significant difference of the activity coefficient of the two morphs of *P. exigua*.

Figure 10 also shows the difference in turn-over times of the two morphs i.e. that the green morph, on average, turned faster than the mottled morph.

The results of a GLM, which includes all the factors which contribute to righting time are summarized in Table 3. It shows that dissolved oxygen, temperature and morph/form all play a significant role in righting time, all having significant p-values (less than 0.05). From these results it can be concluded that the green morph of *P. exigua* does have a faster righting time than the mottled morph. However this experiment did not find out which variable is more important in determining righting time, as both dissolved oxygen and temperature played significant roles in the righting times.

6. Acknowledgements

I would like to thank Charles Griffiths and Katherine Dunbar for helping with the planning and setting up of the experiment and their input with writing up the project, Clement Arendse and Ryan Blanchard for ~~there~~ their help with data collection and writing up the project, Mark Burman for aid with statistical analyses and George Du Plessis for aid with set up and running of the experiment.

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