# Statistical Methods

Data was stored in Microsoft Excel (V16.0.17126.20126) and analysed and plotted in R (R Core Team, 2023).

The data were tested for normality (Shapiro-Wilk’s test using *stats* package in R) (Shapiro & Wilk, 1965) and homogeneity of variance (Levene’s test using *car* package in R) (Levene, 1960) and if these assumptions were met then a one-way ANOVA was conducted to assess the dietary and temperature and treatment factor effects (on somatic growth rate, gonad colour (CIE L\*a\*b values), gonad development and gonad index (GSI)). Where significant results were found, Tukey’s pairwise comparisons were conducted to determine treatment differences.

If data failed to meet ANOVA assumption of normality following either a log or square root transformation, then a nonparametric Kruskal Wallis test (using the *stats* package in R) (Kruskal & Wallis, 1952) was conducted (survival rate, if possible, state which datasets). Where significant differences occurred, a Dunn’s (reference) post-hoc comparison test was conducted to identify the treatment differences.

If data had a normal distribution but failed to meet the assumption of homogeneity, then a Welch ANOVA (reference) test was conducted. Where significant differences occurred, a Games-Howell (reference) post-hoc comparison test was conducted to identify the treatment differences.

# Results

## Survival and somatic growth

There were no significant differences in test diameters (mm) *P. angulosus* between treatments at the start of the experiment (χ2= 12.164, df = 7, p = 0.095; Figure 1a)

Somatic growth rates were measured in terms of specific growth rate (SGR) (% growth day-1) for test diameter (SGRsize) and whole animal wet mass (SGRweight). The SGRweight of *P. angulosus* fed kelp (*Ecklonia maxima*) was significantly lower than the mixed and formulated diets (F = 38.23, df = 3, p-value < 0.001) and treatments (F = 17.9,

Significant differences in survival rate were found between diets (χ2= 10.7, df = 3, p-value < 0.05) and treatments (χ2= 14.511, df = 7, p-value < 0.05; Figure \*) in week 8 of the experiment. The kelp dietary treatment had the lowest survival rate (%) at this timepoint (mean ± se) (76.32 ± 8.18).

A screenshot of a graph

Description automatically generated

The provision of different dietary (f: formulated, k: kelp, m: mixed, u: ulva) and temperature (a: ambient, w: warm) treatments had a significant impact on the in terms of the wet weight of the whole urchin and the size of the urchin test diameter (SGRdiam). The influence of different dietary treatments significant impacted SGRweight after 4 weeks (F = 38.23, df = 3, p < 0.001, Figure \*). A post hoc Tukey test showed that in week 4 the formulated dietary treatment group, which had the fastest SGRweight (mean ± se) (fa: 0.23 ± 0.03 %; fw: 0.20 ± 0.02 %), and the kelp dietary treatment group, which had the slowest SGRweight (mean ± se) (ka: -0.03 ± 0.03 %; kw: -0.10 ± 0.04%), were significantly different to one another and to the other dietary treatment groups. The negative SGRweight values for the kelp dietary treatment group indicated that, on average, the urchins fed kelp had lost weight over the first 4 weeks of the experiment. There was a significant increase in SGRweight for the kelp diet and warm temperature (kw) treatment group between week 4 and week 8 (kwweek8: 0.18 ± 0.09%) such that by week 8 there were no longer significant differences in SGRweight between dietary (F = 1.97, df = 3, p-value >0.05) or temperature (F = 2.91, df = 1, p-value > 0.05) treatment groups. Numerous urchins in the kelp dietary treatment group presented severe spine loss (Figure 1\*) and were therefore removed from the tanks and euthanized as a result of poor health and the impact it may have on the other urchins in the tank and overall water quality.

Significant differences in survival rates were found between dietary treatments after 9 weeks (χ2= 20.332, df = 3, p-value < 0.001). A post hoc Dunn’s test showed that after 9 weeks, due to the high degree of unhealthy urchins within the kelp dietary treatment, the kelp dietary treatment had a significantly lower survival rate than all other dietary treatment groups (mean ± se) (ka: 67.11 ± 6.58%; kw: 65.79 ± 6.26%) at p < 0.05. Due to animal ethics concerns, the kelp dietary treatment was suspended after week 9, all urchins subjected to the kelp dietary treatment were removed from the experiment and euthanized. Kelp was also removed from the mixed dietary treatment feeding regime, changing the regime to a rotation of *Ulva* and formulated feed on a weekly basis from week 10 onwards.

After the removal of kelp from the mixed dietary treatment feeding regime there was a significant increase in SGRdiam (ma: t = -8.611, df = 2,…) by week 13.

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| --- | --- | --- | --- | --- |
| **Diet** | **Temperature** | **Treatment** | **Mean urchin test diameter (mm)**  **(mean ± se)** | **Mean urchin wet mass (g)**  **(mean ± se)** |
| formulated | ambient | fa | 32.91 ± 0.83 | 16.75 ± 0.77 |
| formulated | warm | fw | 35.61 ± 0.73 | 18.92 ± 0.75 |
| mixed | ambient | ma | 32.52 ± 0.85 | 15.00 ± 0.70 |
| mixed | warm | mw | 33.81 ± 0.90 | 16.91 ± 0.87 |
| ulva | ambient | ua | 33.71 ± 0.88 | 16.75 ± 0.83 |
| ulva | warm | uw | 33.80 ± 0.81 | 17.04 ± 0.79 |
| kelp | ambient | ka | 34.19 ± 0.96 | 17.83 ± 0.95 |
| kelp | warm | kw | 32.40 ± 0.72 | 15.70 ± 0.72 |

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| --- | --- | --- | --- | --- | --- | --- |
| **Week** | **Diet** | **Temperature** | **Treatment** | **SGRweight (%)**  **(mean ± se)** | **SGRdiam (%)**  **(mean ± se)** | **Survival rate (%)**  **(mean ± se)** |
| 4 | formulated | ambient | fa | 0.24 ± 0.03 | 0.25 ± 0.12 | 100.00 ± 0.00 |
| 4 | formulated | warm | fw | 0.20 ± 0.02 | -0.01 ± 0.02 | 100.00 ± 0.00 |
| 4 | mixed | ambient | ma | 0.12 ± 0.03 | 0.12 ± 0.06 | 100.00 ± 0.00 |
| 4 | mixed | warm | mw | 0.10 ± 0.04 | -0.09 ± 0.06 | 100.00 ± 0.00 |
| 4 | ulva | ambient | ua | 0.05 ± 0.02 | 0.03 ± 0.04 | 98.68 ± 1.32 |
| 4 | ulva | warm | uw | 0.14 ± 0.03 | 0.01 ± 0.04 | 100.00 ± 0.00 |
| 4 | kelp | ambient | ka | -0.03 ± 0.03 | 0.00 ± 0.03 | 100.00 ± 0.00 |
| 4 | kelp | warm | kw | -0.10 ± 0.04 | 0.08 ± 0.15 | 100.00 ± 0.00 |
| 8 | formulated | ambient | fa | 0.37 ± 0.00 | 0.08 ± 0.02 | 92.11 ± 3.40 |
| 8 | formulated | warm | fw | 0.23 ± 0.05 | 0.14 ± 0.11 | 98.68 ± 1.32 |
| 8 | mixed | ambient | ma | 0.32 ± 0.03 | -0.02 ± 0.06 | 97.37 ± 2.63 |
| 8 | mixed | warm | mw | 0.26 ± 0.04 | 0.16 ± 0.04 | 100.00 ± 0.00 |
| 8 | ulva | ambient | ua | 0.27 ± 0.06 | 0.06 ± 0.05 | 88.16 ± 3.95 |
| 8 | ulva | warm | uw | 0.16 ± 0.03 | 0.10 ± 0.09 | 98.68 ± 1.32 |
| 8 | kelp | ambient | ka | 0.15 ± 0.09 | 0.05 ± 0.08 | 76.32 ± 8.18 |
| 8 | kelp | warm | kw | 0.18 ± 0.09 | -0.00 ± 0.09 | 76.32 ± 8.18 |
| 13 | formulated | ambient | fa | 0.20 ± 0.01 | 0.18 ± 0.05 | 90.79 ± 3.31 |
| 13 | formulated | warm | fw | 0.09 ± 0.03 | 0.11 ± 0.06 | 98.68 ± 1.32 |
| 13 | mixed | ambient | ma | 0.30 ± 0.03 | 0.33 ± 0.05 | 93.42 ± 6.58 |
| 13 | mixed | warm | mw | 0.22 ± 0.03 | 0.30 ± 0.04 | 98.68 ± 1.32 |
| 13 | ulva | ambient | ua | 0.25 ± 0.03 | 0.23 ± 0.08 | 84.21 ± 5.26 |
| 13 | ulva | warm | uw | 0.14 ± 0.02 | 0.14 ± 0.07 | 98.68 ± 1.32 |
| 18 | formulated | ambient | fa | 0.11 ± 0.07 | -0.03 ± 0.03 | 88.16 ± 4.49 |
| 18 | formulated | warm | fw | -0.02 ± 0.06 | -0.04 ± 0.03 | 98.68 ± 1.32 |
| 18 | mixed | ambient | ma | 0.27 ± 0.05 | -0.03 ± 0.03 | 90.79 ± 7.56 |
| 18 | mixed | warm | mw | 0.19 ± 0.03 | -0.02 ± 0.04 | 98.68 ± 1.32 |
| 18 | ulva | ambient | ua | 0.17 ± 0.04 | 0.04 ± 0.03 | 81.58 ± 4.59 |
| 18 | ulva | warm | uw | 0.15 ± 0.05 | 0.07 ± 0.04 | 97.37 ± 2.63 |
| 23 | formulated | ambient | fa | -0.00 ± 0.02 | 0.15 ± 0.03 | 85.53 ± 5.84 |
| 23 | formulated | warm | fw | 0.04 ± 0.03 | 0.06 ± 0.04 | 96.05 ± 2.52 |
| 23 | mixed | ambient | ma | 0.14 ± 0.01 | 0.18 ± 0.02 | 90.79 ± 7.56 |
| 23 | mixed | warm | mw | 0.18 ± 0.02 | 0.04 ± 0.03 | 98.68 ± 1.32 |
| 23 | ulva | ambient | ua | 0.03 ± 0.03 | 0.08 ± 0.05 | 81.58 ± 4.56 |
| 23 | ulva | warm | uw | 0.12 ± 0.05 | 0.06 ± 0.03 | 97.37 ± 2.63 |

