Assessing the potential of the unexploited South African Cape sea urchin, *Parechinus angulosus*, as a new aquaculture species.

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Declaration

I declare that this project is my own, unaided work and has not been previously submitted, in whole or in part, for the award of any degree. Where use has been made of the research of others, it has been duly acknowledged in the text. This project was carried out in the Department of Biological Sciences under the supervision of Dr Marissa Brink-Hull, Dr Brett Macey, and Professor John Bolton.

Signed by candidate

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Abstract

Sea urchins are harvested for their gonads, otherwise known as “uni”, that have a high market value, particularly in Asian countries such as Japan and China. Over the last few decades, the market for ‘uni’ has steadily expanded. Current and future demand for sea urchin products cannot be met by the sea urchin fishery alone, presenting a commercial opportunity for unexploited sea urchin species with marketable gonad products.

A symbiotic relationship exists in nature between the Cape urchin *Parechinus angulosus* and the early developmental stages of South Africa’s most lucrative aquaculture product, the South African abalone *Haliotis midae*. Previous work has shown that supplementing hatchery-produced juvenile abalone with Cape sea urchin faecal matter benefits juvenile abalone growth and enhances survival. Given that abalone farms have well-established trade routes to Asian markets, where urchin demand is high, the Cape urchin could be traded as an additional value-added product while improving juvenile abalone health and growth. Currently, the Cape urchin is unexploited, and no literature is available on their market potential.

Therefore, the aim of this study was to assess the feasibility of the Cape urchin as a new South African aquaculture species. To achieve this, Cape urchins (30 – 50 mm test diameter) were collected in the intertidal and shallow subtidal on a rocky shore in front of the Department of Forestry, Fisheries, and the Environment (DFFE) Marine Research Aquarium (MRA) in Sea Point, Cape Town (33° 55' 6.492" S, 18° 22' 52.572" E). The animals were reared in a flow-through experimental system at the MRA under two temperature treatments and four dietary treatments over a period of 26 weeks. The urchins were cultured in either ambient incoming seawater (mean ± SEM; 15.56 ± 0.07 ºC) or heated seawater (18.94 ± 0.03 ºC). Two macroalgal diets (*Ulva lacinulata* (U) and *Ecklonia maxima* (K)), one formulated feed diet (with 16% (w/w) inclusion of dried *Ulva*) (F) and a combination of the aforementioned diets rotated on a weekly basis to form a mixed diet feeding regime (M) were tested. Somatic growth, feed consumption, feed conversion ratio, gonad somatic index (GSI) and gonad colour were assessed across the dietary and temperature treatments.

The results from this study showed that the kelp *Ecklonia maxima* is not a suitable feed for this species. Urchins fed *E. maxima* had poor survival (< 70 %), somatic growth (-0.1 – 0 % growth.day^{-1}), and low gonad yield (2 – 3 % GSI). The formulated feed promoted high gonad yield (25 – 30 % GSI), however gonad colour (pale) did not meet market standards. *Ulva lacinulata* promoted favourable (red orange) gonad colour and was an effective feeding stimulant, however gonad yield was low (11 – 13 % GSI). A combination of *U. lacinulata* and formulated feed (mixed diet) promoted marketable (yellow) gonad colour and a high gonad yield (20 – 22 % GSI). The female gonads of *Ulva*-fed urchins were more marketable, having greater redness based on visual assessment and colour (CIE L*a*b*) indices.

The warmer temperature treatment significantly increased the consumption of *U. lacinulata* by urchins in the *Ulva* and mixed dietary treatments (ambient: 1 – 2 g/week per urchin; warm: 2 – 3 g/week per urchin). However, the increased consumption did not correspond with increased weight gain between the temperature treatments. Conversely, the warmer temperature showed a significant reduction in total weight gain over the entire trial period for urchins fed the formulated feed (ambient: 5.34 ± 0.41 g; warm: 3.34 ± 0.36 g), but no effect on feed consumption rate (~ 0.5 g/week per urchin) was observed. Feed conversion ratio (FCR), representing the grams of feed consumed per gram of urchin wet weight gained over the entire trial period, differed significantly between temperatures for all dietary treatments. *Parechinus angulosus* gained significantly more weight for the same "ration" of feed in the ambient temperature (U: 9.81 ± 0.98; M: 2.79 ± 0.23; F: 1.91 ± 0.23 FCR) compared to the warm temperature (U: 16.24 ± 2.37; M: 4.58 ± 0.44; F: 3.02 ± 0.29 FCR). Based on the results of this study, increasing water temperature to ca. 19°C did not benefit growth in this species during gonad enhancement as it had no benefit on the market quality or gonad yield. However, the age of the wild-collected animals used in this study was unknown and increasing the culture water temperature may benefit other developmental stages.

This study has shown that *P. angulosus* can produce marketable quality gonads, in terms of quantity (gonad weight (g) and GSI (%)) and quality (gonad colouration), which could have commercial interest. However, taste and texture are key purchase determinants which influence market value. Full-life cycle grow-out experiments where urchins are maintained at different temperatures and fed different diets, coupled with taste and texture assessments, are required to further assess the commercial potential of this species.

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List of acronyms

ASTRAL	All Atlantic Ocean Sustainable, Profitable and Resilient Aquaculture
AAEC	Aquaculture Animal Ethics Committee
CIE	International Commission on Illumination
DFFE	Department of Forestry, Fisheries and the Environment
DOM	Dissolved Organic Matter
DM	Dry Matter
FCR	Feed Conversion Ratio
GSI	Gonad Somatic Index
IMTA	Integrated Multi-Trophic Aquaculture
MRA	Marine Research Aquarium
POM	Particulate Organic Matter
PC	Protein Content
SGR	Specific Growth Rate

1. Introduction

Current and future demand for sea urchin products cannot be met by the sea urchin fishery alone, presenting a commercial opportunity for unexploited sea urchin species with marketable gonad products. The aim of this study was to assess the feasibility of the South African Cape sea urchin (*Parechinus angulosus*) as a new aquaculture species. Currently, *P. angulosus* is unexploited, and no literature is available on their market potential. By investigating the somatic growth, gonad growth, feed consumption and gonad quality of *P. angulosus* fed different diets and maintained and different temperatures, this study seeks to address this knowledge gap. Additionally, the findings of this study aim to provide insight which may benefit the development of sustainable aquaculture in South Africa through species diversification and integrated multi-trophic aquaculture.

1.1. Global aquaculture

Aquatic foods play a crucial role in ensuring food and nutrition security, particularly for vulnerable coastal populations, by providing accessible and affordable sources of proteins and micronutrients (FAO 2024). Global demand for aquatic foods is projected to increase and the sustainability of fishery resources is a cause for concern as the percentage of fishery stocks within biologically sustainable levels continues to decline (FAO 2024). Sustainable fisheries play a critical role in securing food supply and achieving various United Nations Sustainable Development Goals (SDGs), notably sustainable ocean use (SDG 14), food security (SDG 2), decent work (SDG 8) and sustainable production and consumption (SDG 12) (Troell et al. 2023). Human population growth has contributed to an increase in legal and illegal marine exploitation and subsequent declines in biologically sustainable fishing stocks (Pham et al. 2023). This, coupled with climate change has placed immense pressure on ocean productivity which has a detrimental impact on marine ecosystems. Therefore, novel approaches to marine resource management must be explored (Zhao and Li 2023). The rise of aquaculture as an alternative source of aquatic food products to capture fisheries can provide valuable insights for the development of sustainable new foods (Longo et al. 2019).

Aquaculture has recently surpassed capture fisheries and contributes 51% to global aquatic animal production (FAO 2024). Future projections place aquaculture expansion as a key driver to fill most of the supply-demand gap for aquatic foods. However, aquaculture expansion must prioritize sustainability (FAO 2024). Although the environmental performance of aquaculture has improved over the past 20 years, certain sustainability challenges still require improvement, such as reducing the use of marine resources for feeding farmed species, limiting habitat degradation or improving disease management (Naylor et al. 2021). Climate change, disease outbreaks, market fluctuations, and other disturbances are challenges faced by all food production sectors. Despite the great diversity in farmed aquatic and terrestrial species, there are only a small number of "staple" species that presently dominate production, with 17 out of over 700 farmed aquatic species representing ~ 60% of global aquaculture production (FAO 2024). Growth and resilience of the aquaculture sector through species diversification has become a widely recognised and endorsed development strategy, both in the policy and in scientific communities (Cai et al. 2023). Moreover, tackling unsustainable practices used in aquaculture is also necessary to meet the SDGs highlighted above — as it is now widely recognised that sustainable development (environmental, economic and social) of the aquaculture industry is necessary to meet the growing demand for seafood products.

The high concentrations of dissolved nutrients, particulate organic matter (POM) and uneaten feed in effluent water and the use of wild-caught marine fish stocks for fishmeal in aquafeeds, raises concerns about the sustainable expansion of the aquaculture industry. Aquaculture effluent water may contain large amounts of nutrients, particularly nitrogen and phosphorus, and possibly also other chemicals that are used to prevent or treat diseases of aquatic species (Jegatheesan et al. 2011). This effluent water, when discharged into the environment untreated, can be detrimental to the surrounding environment and human health. Macroalgal aquaculture has been noted for it's potential to extract excess dissolved nutrients from effluent water and act as a carbon sink (Gao and Beardall 2022). Co-culturing macroalgae or other lower trophic level organisms on aquafarms which can make use of the dissolved or POM released by other farmed species acts as a biofiltration system (bioremediation), reducing the ecological impact of aquaculture practices on the environment (Neveux et al. 2018). Integrating multiple trophic levels in aquaculture practices, is a concept commonly referred to as integrated multi-trophic aquaculture.

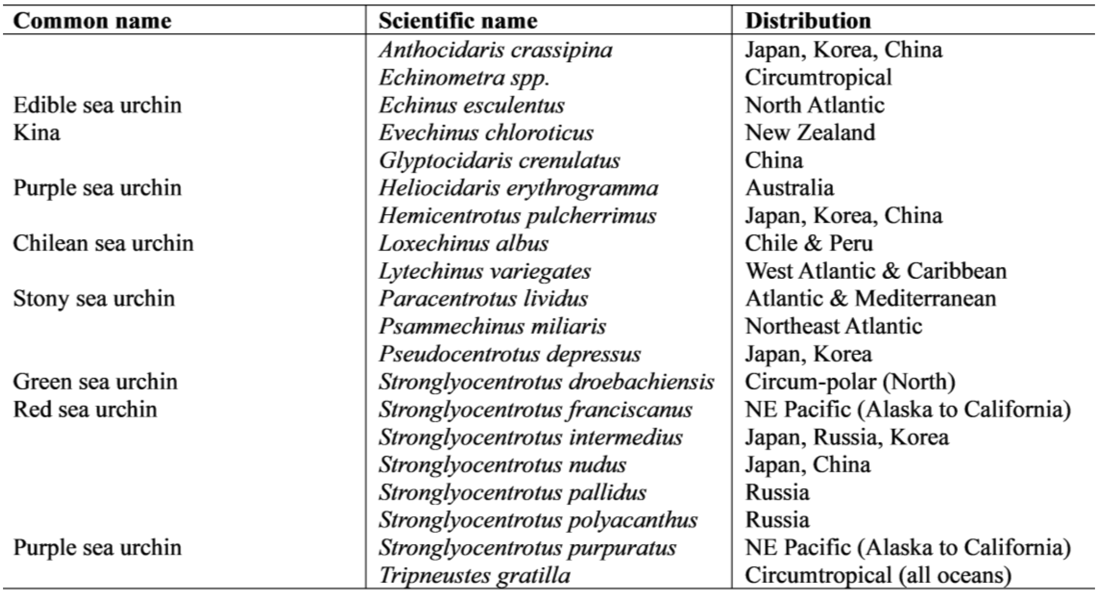
Integrated multi-trophic aquaculture (IMTA) is considered a suitable and sustainable approach to limit aquaculture nutrients and organic matter outputs through bioremediation (Neveux et al. 2018). This farming practice involves two pillars of circularity: nutrient management, and resource use efficiency (Checa et al. 2024). In IMTA systems, uneaten feed and excreted matter (dissolved/particulates) from fed species become food/nutrients for extractive species, such as filter-feeders and seaweeds, creating a balanced ecosystem management approach to aquaculture (FAO 2024). The efficiency of the bioremediation is dependent on the metabolic activities of the organisms involved (Neveux et al. 2018). It is essential that the environment (the contaminants in the effluent water) provide resources which are conducive to the growth of the extractive species (Neveux et al. 2018). The extractive species may also be traded as a commodity in addition to its bioremediation services. Therefore, IMTA encourages species diversification while reducing environmental impact through efficient nutrient and resource management. In summary, IMTA contributes to the societal, environmental and economic sustainability of the aquaculture sector (Chopin et al. 2001, 2012, Barrington et al. 2010, Diana et al. 2013; Checa et al., 2024).

South Africa has successfully implemented IMTA for more than 20 years on abalone (*Haliotis midae*) farms (Bachoo et al. 2023). Fully commercial integrated abalone-*Ulva* systems are in operation and a pilot commercial-scale urchin-*Ulva* IMTA system has been investigated using the collector sea urchin, *Tripnuestes gratilla* (Checa et al. 2024). In both systems the macroalgae *Ulva* is used both as a feed and for the bioremediation of the effluent water to enable partial recirculation of water (Bolton et al. 2009, Checa et al. 2024). These IMTA systems have been shown to increase recirculation (%) of seawater and decrease energy (kWh) consumption in comparison to monoculture systems (Checa et al. 2024). To further develop these IMTA systems additional compatible species should be identified and investigated.

Field experiments have shown that juvenile abalone populations in nature are highly compatible and dependent on co-habitation with the Cape sea urchin (*Parechinus angulosus*), becoming virtually extinct locally in their absence (Day and Branch 2002a). Juvenile abalone prefer to shelter beneath the Cape sea urchin rather than under rocks and crevices, as they gain protection from predators and benefit from an enhanced food supply (Day and Branch 2002a, 2002b). Considering the co-habitation of juvenile abalone and the Cape urchin in natural environments, these species present another opportunity for IMTA given their similar temperature preference and possible symbiotic relationship. However, little is known about the Cape urchin as an aquaculture species.

1.2. The sea urchin market

The aquaculture potential of the Cape urchin is dependent on the marketability of its products (gonads). Sea urchins are harvested for their gonads (roe), otherwise known as “uni”, which is a prized delicacy mostly served in sushi bars and at special occasions, such as wedding banquets and parties, in Japan (Sun and Chiang 2015). Consumption of sea urchin gonads extends globally, with Asia, Europe (France, Italy & Spain), South America (Chile) and North America being notable consumers (Stefánsson et al. 2017). The ‘uni’ market is driven by Japan, which accounts for approximately 75% of global demand (Bennett et al. 2024). However, supply is dominated by only a relatively small number of exploited species (Figure 1), with only 20 out of the approximately 940 species distributed worldwide commercially exploited (Follo and Fautin 2024).



Common name	**Scientific name**	**Distribution**
	Anthocidaris crassipina	Japan, Korea, China
	Echinometra spp.	Circumtropical
Edible sea urchin	*Echinus esculentus*	North Atlantic
Kina	*Evechinus chloroticus*	New Zealand
	Glyptocidaris crenulatus	China
Purple sea urchin	*Heliocidaris erythrogramma*	Australia
	Hemicentrotus pulcherrimus	Japan, Korea, China
Chilean sea urchin	*Loxechinus albus*	Chile & Peru
	Lytechinus variegates	West Atlantic & Caribbean
Stony sea urchin	*Paracentrotus lividus*	Atlantic & Mediterranean
	Psammechinus miliaris	Northeast Atlantic
	Pseudocentrotus depressus	Japan, Korea
Green sea urchin	*Stronglyocentrotus droebachiensis*	Circum-polar (North)
Red sea urchin	*Stronglyocentrotus franciscanus*	NE Pacific (Alaska to California)
	Stronglyocentrotus intermedius	Japan, Russia, Korea
	Stronglyocentrotus nudus	Japan, China
	Stronglyocentrotus pallidus	Russia
	Stronglyocentrotus polyacanthus	Russia
Purple sea urchin	*Stronglyocentrotus purpuratus*	NE Pacific (Alaska to California)
	Tripneustes gratilla	Circumtropical (all oceans)

Figure 1. Sea urchin species with a major contribution to the modern global fishery (Stefánsson et al. 2017).

Over the last few decades, the market for ‘uni’ has steadily expanded and is unlikely to decline in the future (Sun and Chiang 2015, Onomu et al. 2020). Increased demand and decreased supply have influenced the value of sea urchin roe. Sea urchin gonads are sold live, chilled, frozen, or processed (dried, salted, pastes or in brine).

Sea urchin product (live and processed) prices are dependent on several factors including appearance (colour and texture), origin (species, region of harvest, supplier), palatability (flavour, texture), demand, distribution, form and processing (Cyrus et al. 2014, Stefánsson et al. 2017). Live sea urchin products are in highest demand and fetch the highest prices (Sun and Chiang 2015).

Until 1987, Japan was the largest global harvester of sea urchin and since then Japanese harvests have sharply declined due to overfishing. To meet the increasing demand for urchin products, Japan became more dependent on imports (Sonu 2017). Key suppliers of urchin products to Japan are Chile, Russia, North Korea, the United States and Canada (Stefánsson et al. 2017). In 2016, Japan's urchin product imports were valued at approximately 183 million USD (Stefánsson et al. 2017). Import price per kilogram has steadily increased from about 124 USD in 2016 to 200 USD in 2022 (Selina Wamucii 2022). The steadily increasing demand and high commercial value for sea urchin gonads has led to overfishing on a global scale. Wild sea urchin stocks have declined and, in some cases, collapsed in many countries where appropriate harvest management strategies have not been put in place (Micael et al. 2009, Johnson et al. 2012, Stefánsson et al. 2017) (

Figure 2).

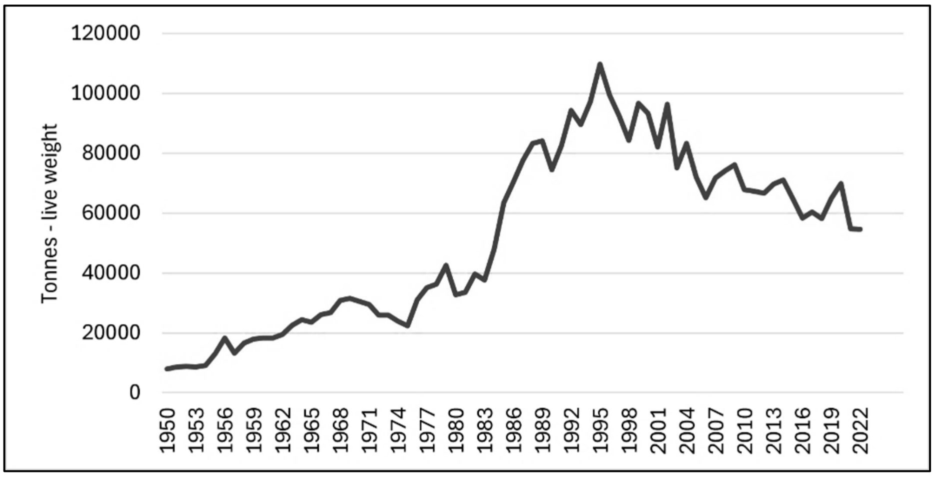


Figure 2. Global landings of sea urchins between 1950 and 2022 (FAO FishStatJ 2024)

In addition to harvesting pressure, pathogens, rising temperatures, predators and climate change effects on ocean chemistry (ocean acidification) threaten sea urchin populations (Yeruham et al. 2015, 2020). Sea urchins inhabit and functionally control the structure of a plethora of shallow marine communities worldwide including kelp forests, sea grass beds, and coral reef ecosystems (Steneck 2020). Ocean acidification results in reduced concentrations of minerals needed for calcification in planktonic and benthic life stages of sea urchins (Byrne and Hernández 2020). Reduced calcification capacity stunts the growth and development of sea urchin species which are not adapted to naturally acidified habitats (Byrne and Hernández 2020). On Caribbean reefs, urchins control algae growth and increase surface area for coral recruitment. A mass mortality event of *Diadema antillarum* occurred in 1983 and 2022 due to pathogens (Levitan et al. 2023), with the loss of these sea urchins subsequently threatening coral recruitment within the relic coral community (Levitan et al. 2023). Coral reef recovery projects exist in Caribbean and Pacific regions, which utilize sea urchin restoration as a method to speed up coral reef recovery (NOAA Fisheries 2023, Hylkema 2024). Contrarily, in some regions (e.g., coastal areas of the Pacific Ocean) an increase in sea urchin populations has occurred, which threatens the health of kelp forest ecosystems (Harris and Tyrrell 2001, Filbee-Dexter and Scheibling 2014) (Figure 3). In such cases, urchins (e.g. *Strongylocentrotus droebachiensis*) form feeding fronts which decimate kelp populations to the point of a regime shift from an algal-dominated ecosystem to an urchin-dominated alternative stable state known as an “urchin barren” (Filbee-Dexter and Scheibling 2014).

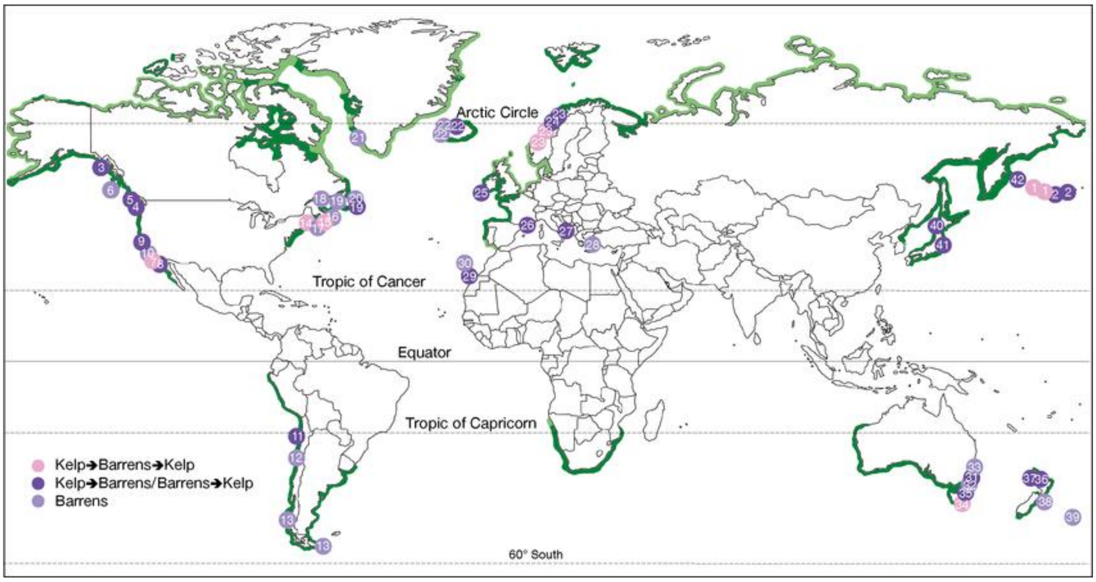


Figure 3. Global occurrence of sea urchin barrens documented throughout the range of kelp. Numbered locations indicate areas where urchin barrens have been documented. Observed range (dark green) and potential range (light green) of kelp is also shown (adapted from Filbee-Dexter and Scheibling 2014).

Urchins harvested from urchin barren regions are often malnourished, do not have sufficient or good quality gonads and therefore are not marketable (Angwin et al. 2022). Gonad enhancement of urchins collected from barrens has been shown to be successful through echinoculture (Barker et al. 1998, Angwin et al. 2022, Suckling et al. 2022). Thus, echinoculture has the potential to be used as a tool for both restoration (of kelp forest and coral reef ecosystems) and urchin market development.

1.3. *Parechinus angulosus*

The Cape urchin, *Parechinus angulosus* (Leske, 1778), an endemic southern African species belonging to the Parechinidae family, does not form urchin barrens as far as anyone is aware (Filbee-Dexter and Scheibling 2014) (Figure 3). *Parechinus angulosus* is widely distributed from Lüderitz (Namibia) to Umhlali (Kwa-Zulu Natal, South Africa) (Figure 4). The species has high densities around the Cape Peninsula (Fricke, 1980), a region with a strong seawater temperature gradient (~12ºC – 17 ºC) (Muller et al. 2012, Smit et al. 2013)

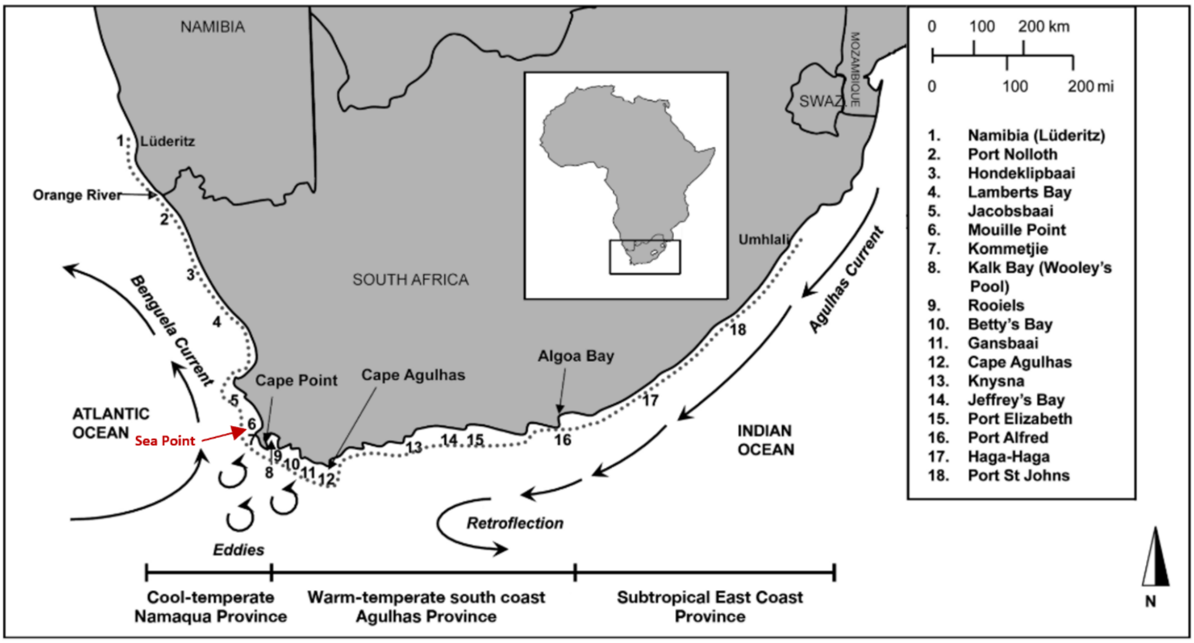


Figure 4. Sampling localities for a phylogeographic study by Muller et al (2012) along the southern African coastline covering the range of *Parechinus angulosus* (grey dotted line). The three marine biogeographic provinces and the main directions of oceanic currents are indicated. (adapted from Muller et al., 2012).

The western distributions of *P. angulosus* overlap with extensive kelp forests formed by *Ecklonia maxima* (south-west) and *Laminaria pallida* (west) between Cape Agulhas and Namibia (Morris and Blamey 2018). The generally turbulent sea conditions in the region produce kelp detritus and provide an ample supply of organic matter for the urchins, reducing the likelihood of active grazing (Fricke 1979). Although Cape urchins can climb up and actively graze on the kelp stipes under calm conditions (Fricke 1979, Morris and Blamey 2018) this behaviour is uncommon. Additionally, *Ecklonia maxima*, the more dominant kelp species, is among the least preferred algae species for *P. angulosus* when considering the 13 most common algae in the kelp beds of the west coast of the Cape Peninsula (Anderson and Velimirov 1982). East of Cape Agulhas there is very little kelp, with most of the seaweed biomass being red algae (Bolton and McQuaid 2023). *P. angulosus* density decreases northwards, along the east coast, in relation to increased water temperature (Greenwood and Bennett 1981).

Corresponding with oceanic currents, two distinct geographic genetic groupings of *P. angulosus* were found based on geneflow analyses, parsimony haplotype networks for both nDNA and mtDNA data, and a mtDNA Bayesian analyses of population structure (Muller et al. 2012). The genetic separation of this species is in the vicinity of Cape Point, a recognised biogeographic barrier (Muller et al. 2012) and possible driver of genetic differentiation in broadcast spawning organisms with a planktonic larval phase (Teske et al., 2011). The study by Muller et al (2012), identified a clear genetic differentiation between urchins sampled in the cool temperate Namaqua province population (west of Cape Point) and urchins sampled in the warm temperate Agulhas and subtropical East Coast provinces (Figure 4). Additionally, outlier/adaptive single nucleotide polymorphism (SNP) markers identified environmental drivers (sea surface salinity & air temperature) for genomic variation between *P. angulosus* populations in these regions to assess population structure (Nielsen et al. 2020). Sea Point, the area from which the urchins sampled for this study were collected, falls within the cool temperate Namaqua province (Figure 4).

Cape urchins are a key trophic intermediate in South African kelp bed ecosystems as they control the rate of kelp settlement by grazing on young sporophytes and kelp debris (Fricke 1979). In doing so, their messy feeding, shredding and egestion processes (Yorke et al. 2019) makes detritus more accessible to benthic consumers, such as abalone *Haliotis midae*. Cape urchins are prey, although not preferentially, for rock lobsters, *Jasus lalandii* (Van Zyl et al. 2003). South-eastward shifts of rock lobster distributions have caused severe declines in Cape urchin populations which has ecological consequences and economically substantial impacts on commercial harvests of abalone *H. midae* (Blamey et al. 2010). The Cape urchin assists in maintaining the balance and productivity of kelp forest ecosystems in South Africa. Their ecological significance is clear, but their economic and cultural significance requires further study.

The Cape urchin is unexploited commercially and does not have a market value, however a local company, "*Veld and Sea"*, which promotes and offers coastal foraging workshops, harvests Cape urchins seasonally as part of the experience, and the gonad tissue colour of wild harvested Cape urchins appears to have marketable qualities (Figure 5).

Figure 5. Image of wild Cape urchin, *P. angulosus*, harvested by a company "Veld and Sea" which offers wild coastal food foraging workshops (Connell 2021).

1.4. Key considerations for echinoculture

Due to the high degree of importance placed on product presentation in the sushi market, the quality of the sea urchin gonad is a primary driver for their market value (Robinson et al. 2002, Teck et al. 2018). Gonad quality is determined by several factors including colour, texture, taste, and gonad somatic index (GSI) (Cyrus et al. 2015a, 2015b). Gonads that are large, contain few to no gametes, have a firm texture and are bright yellow or orange in colour, are regarded as the most commercially valuable (Robinson et al. 2002, Cyrus 2013). Developing and refining culturing conditions is necessary for the establishment of a commercial sea urchin aquaculture (echinoculture) industry. Culture temperature and diet are two important factors to consider in echinoculture as these affect sea urchin growth and product quality (Kennedy et al. 2007, Siikavuopio et al. 2012, Prato et al. 2018, Lourenço et al. 2020, 2021, Onomu et al. 2020), thereby impacting profit margins for farms.

Water temperature is one of the most important factors governing metabolic processes in poikilothermic ("cold-blooded") animals, such as sea urchins (Siikavuopio et al. 2012). To effectively and profitably culture sea urchins, it is critically important to understand the effects of water temperature on growth rates and animal health (Siikavuopio et al. 2012). Cape urchins inhabit almost the entire coastline of South Africa (Figure 4) across a wide temperature range. Their distribution ranges from the cool temperate northern west coast (monthly mean seawater temperatures 11.8 – 13.2°C) to the subtropical east coast (monthly means 21.9 – 24.1°C) (temperature data from Smit et al. 2013). This indicates that Cape urchins have a high temperature tolerance, an important consideration for sustainable echinoculture.

Sensory properties of sea urchin gonads (colour, taste, and texture) have been shown to be highly influenced by diet (Watts et al. 1998, Pearce et al. 2003, Suckling et al. 2011, Angwin et al. 2022) and sex (Baião et al. 2021, Lourenço et al. 2022). Optimal gonad tissue colour is critically important for sea urchin market value, depending on the local market preferences this can range from a light yellow to a dark orange or almost red. Lighter, pale coloured or dark brown gonads are not as desirable to the market (Robinson et al. 2002). The sought after colour in the sea urchin gonad is primarily due to the xanthophyll pigment echinenone (Griffiths and Perrott 1976), which is synthesised from carotenoids (beta-carotene) by the gut (Symonds et al. 2007) and stored in the gonad (Hagen et al. 2008) of the sea urchin. Carotenoids (carotenes and xanthophylls) are naturally occurring pigments of plant origin which are important for sea urchin nutrition, egg production and development and biological functioning (Suckling et al. 2020). Urchin gonad colour is a function of the carotenoid content of gonad tissue, and substandard colour is a direct result of carotenoid levels being too high (dark) or low (pale) (Hagen et al. 2008).

Dietary beta-carotene directly influences the quality and market value of sea urchin gonads (Robinson et al. 2002). Feeding sea urchins natural diets exclusively may produce market quality gonad products but it is not feasible for all aquafarms. Large quantities of macroalgal biomass are required by sea urchins to meet the nutritional requirements due to the high (80%) water content of seaweeds (Cyrus et al. 2014). Additionally, seaweeds may have seasonal variability in quality/protein content and continuous harvesting could potentially overexploit natural macroalgal populations. Formulated feeds are more efficient than natural diets when considering feed conversion ratios (weight of feed required for production of gonads) of macroalgal and formulated feeds (Cyrus et al. 2014). Moreover, formulated feeds have a long shelf life allowing for global distribution and increased accessibility.

Previous literature has shown that sea urchins fed formulated diets (manufactured feeds) have enhanced gonad yield in comparison to urchins fed natural diets, primarily due to the higher protein levels in formulated feeds (Pearce et al. 2003, Shpigel et al. 2005, Cyrus et al. 2014). However, traditional formulated feeds are less palatable than natural (macroalgal) diets (Robinson et al. 2002, Cyrus et al. 2015b) and produce lighter, pale coloured gonads which are not desirable to 'uni' markets as they lack the naturally occurring pigments (carotenoids) present in macroalgae which sea urchins utilise for their gonad tissue colouration (Lawrence 1975, Barker et al. 1998, Grosjean et al. 1998, Watts et al. 1998, Robinson et al. 2002, Pearce et al. 2003, Suckling et al. 2011, Cyrus et al. 2015a; Brink-Hull et al., 2022b). Gonad colour produced by formulated feeds can be significantly improved through the addition of concentrated pigments (Robinson et al. 2002, Pearce et al. 2003) or by incorporating macroalgae into the feed formulation (Cyrus et al. 2015b). The addition of pigments into formulated diets has been shown to improve somatic growth of urchin test (diameter) (Kennedy et al. 2007). Including macroalgae, specifically the green seaweed *Ulva*, in formulated diets acts as a feeding stimulant, improving consumption rate, digestibility, and palatability of the feed (Cyrus et al. 2015b, Addis et al. 2023), while also improving the colour of the gonad.

A study by Baião et al., (2021) explored chef's perceptions of *Paracentrotus lividus* sea urchin products and found that male urchins were generally linked with undesirable intrinsic attributes having a repugnant appearance, pale colour, and bitter, acidic flavour. Conversely, preferable attributes were associated with female urchins, having an appealing appearance, bright-orange colour, and sweet and fresh sea flavour. Females have a higher capacity for carotenoid storage and echinenone synthesis (Lourenço et al. 2022) as successful oocyte development requires higher quantities of lipid and fatty acid reserves than the development of highly mobile sperm (Díaz de Vivar et al. 2019). However, sex-specific differences in gonad tissue quality (taste and colour) are not as pronounced in all sea urchin species e.g., *Tripneustes gratilla* (Cyrus 2013).

The reproductive state of the urchin impacts the density of gametes and in turn the commercial value of the sea urchin gonads. The price differential paid for sea urchin roe across varying reproductive stages can be substantial (Teck et al. 2018) with the most marketable urchin gonads considered to be those which contain little to no gametogenic activity (Cyrus et al. 2014). The nutritive phagocytes of sea urchin gonads store nutrients such as protein, lipid and carbohydrates for use during gametogenesis (Díaz de Vivar et al. 2019). Ideally, urchin gonads should be harvested when the nutritive phagocytes have attained the greatest degree of mass increase (growing or pre-mature gonad development stages, see Figure 6) before gametogenic differentiation (mature gonad development stage, see Figure 6) begins (Powell et al. 2020). Histological analyses are widely used in echinoculture to determine the amount of gametogenic activity in urchin gonads (Figure 6). Research that develops understanding and manipulates gonad development and tissue colouration is important for the commercial development of the sea urchin aquaculture industry.

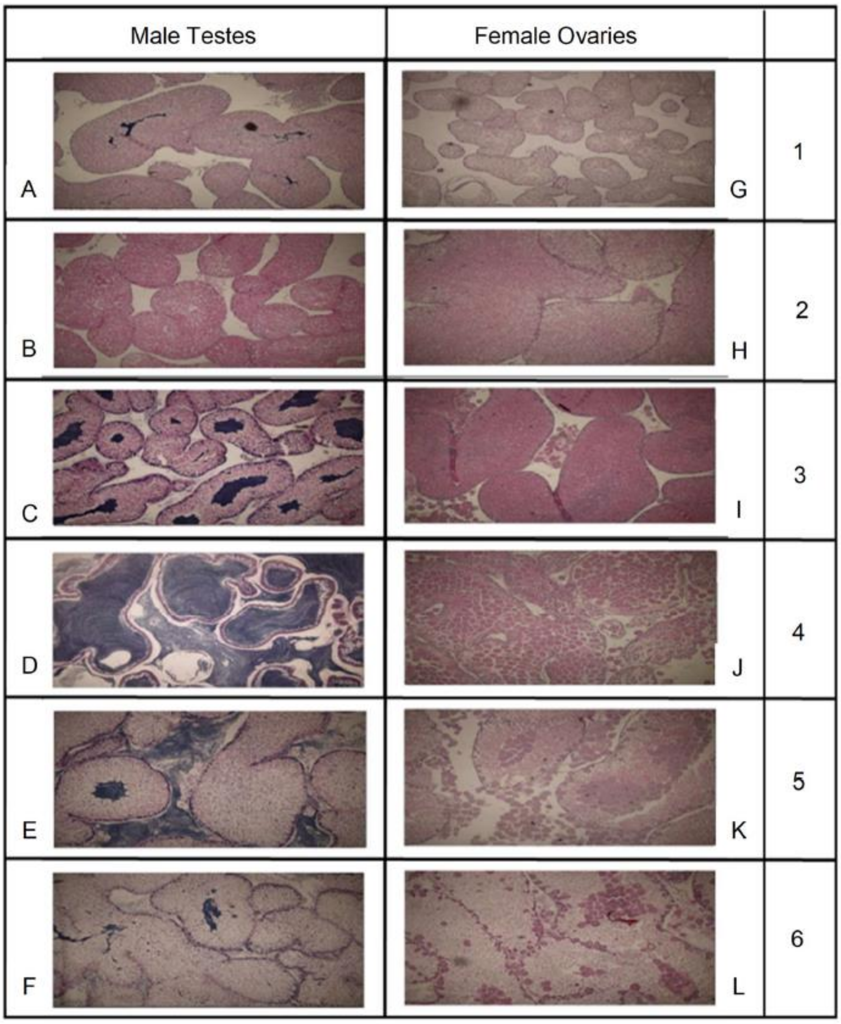


Figure 6. Gonad histology of male (A-F) and female (G-L) *Tripneustes gratilla* gonads in different states of gonad development (1 - Recovery; 2 - Growing; 3 - Premature; 4 - Mature; 5 – Partly spawned; 6 - Spent) (Brink 2020 adapted from Cyrus 2013).

1.5. Research rationale

Current and future global demand for urchin products cannot be met by the sea urchin fishery alone (Asia et al. 2012, Onomu et al. 2020). The decreasing supply and unmet demand in sea urchin markets presents an opportunity for unexploited sea urchin species with good quality gonad products to enter the market (Stefánsson et al. 2017). This has led to increasing interest in sea urchin aquaculture, also known as "echinoculture" (Hagen 1996, Robinson et al. 2002, Lawrence 2007, Siikavuopio et al. 2012, Cyrus et al. 2014, 2015a, Onomu et al. 2020).

The collector sea urchin, *T. gratilla* (Linnaeus, 1758), a sought-after species on the sea urchin market, is distributed on the eastern coast of Southern Africa. This species has been identified as a species with potential for aquaculture production in South Africa, due to its fast growth rate and marketable roe production (Scholtz et al. 2013, Cyrus et al. 2015a, Brink et al. 2018, Onomu et al. 2020, Brink-Hull et al. 2022b). *Tripneustes gratilla* is typically cultured at a temperature of 24 – 25 °C and has been observed to favour the green seaweed *Ulva* over other macroalgal feeds including *Ecklonia maxima*, *Porphyra capensis* and *Gigartina polycarpa* (Cyrus et al. 2014, 2015b, Brink-Hull et al. 2022b). *Ulva*, also known as sea lettuce, is a genus in the Chlorophyta phylum with a wide range of commercial applications (Bachoo et al. 2023). Formulated feeds for *T. gratilla* have incorporated *Ulva* to act as a natural feeding stimulant as well as source of beta-carotene, which aids marketable gonad colour production (Cyrus et al. 2014, 2015b). Formulated feeds supplemented with 20% dried *Ulva* has been shown to produce market-quality gonads and high gonad yields (~20 – 25% GSI) for gonad enhancement as well as full life cycle grow-out of *T. gratilla* in South Africa (Cyrus et al. 2014, 2015a, 2015b). The feasibility of producing this warm-water species is limited however to eastern regions of the South African coastline with warm seawater temperatures or to farms which can afford the high heating costs for optimal growth of the species in a temperate region.

Many of the successful existing aquafarms in South Africa cultivate abalone, *Haliotis midae*. These land-based farms are mostly situated west of Cape Agulhas, the southernmost point of Africa (Bolton et al. 2009). These south and south-west regions of the coast, provide to the optimum temperature range (12 – 20ºC) for abalone cultivation (Vosloo and Vosloo 2010). Abalone is produced primarily for export to Asia and has a high product value, promotes employment in coastal communities that previously relied on wild fisheries, and is one of the species with the highest production volumes when compared to other South African aquaculture products (Britz and Venter 2016). Abalone aquaculture has a 76% share of the overall value generated by the South African aquaculture sector (Britz and Venter 2016). Abalone are generally fed formulated feeds with the addition of seaweeds, either freshly harvested kelp (*E. maxima*) (when available) and/or aquacultured *Ulva* (Rothman et al. 2020). *Ulva* has been cultivated commercially in integrated seaweed-abalone aquaculture farms in South Africa since 2002 (Bachoo et al. 2023). *Ulva* is used as a feed for abalone and for bioremediation, removing dissolved nutrients from abalone effluent and enabling partial water recirculation (Bolton et al., 2009). This is an example of IMTA, which represents a more sustainable farming method than monoculture as the by-products from one species acts as an input for another.

The Cape urchin is an edible species with a similar preferred temperature range (12 – 20 °C) to abalone (Fricke 1980, Day and Branch 2002a, 2002b). Juvenile abalone commonly occur underneath Cape urchins in nature (Day and Branch 2000, 2002a, 2002b). Microalgae growing under rocks and crevices is insufficient to meet the dietary requirements of juvenile abalone (Day and Branch 2002a, 2002b). By sheltering beneath urchins rather than under rocks and crevices, juvenile abalone benefit from food supplied by the Cape urchin (shredded and egested macroalgae) while being shielded from predation (Day and Branch 2002a).

Previous studies assessing the use of Cape urchin faecal matter as a supplementary feed for juvenile abalone found enhanced growth rates of juvenile abalone (Cloete 2022). Co-culturing juvenile abalone and the Cape urchin has the potential to improve juvenile abalone health through the trophic transfer of microbial communities (Brink-Hull et al. 2022a). Considering the potential symbiotic relationship that exists between the Cape urchin and abalone, and the well-established trade routes between abalone farms and Asian markets, they could be co-cultured on existing abalone farms and the Cape urchin could be traded as an additional value-added product. Moreover, research has shown that *Paracentrotus lividus*, a species in the same Parechinidae family has potential to thrive in open-water IMTA with salmon farms (Cook and Kelly 2007). Cape urchins may therefore be suitable for open-water IMTA with mussel farms as these are situated on the west coast within natural distribution ranges.

The introduction of the Cape urchin as an aquaculture species would diversify the South African aquaculture sector and contribute towards improving sustainability in the sector. However, the market potential for Cape urchin products has not been investigated. Currently the Cape urchin, *Parechinus angulosus,* is unexploited and no literature is available on their market value. The effects of different temperatures and feeding regimes on the growth performance (somatic and gametogenic), optimal gonad tissue colour and gonadal somatic index (GSI) of this species has not yet been assessed, this project aims to address these knowledge gaps.

1.6. Research aim and objectives

The aim of this study is to assess the feasibility of the Cape urchin, *Parechinus angulosus*, as a new aquaculture species in South Africa.

The objectives of this study were:

1. To assess the effects of four different dietary treatments including *Ulva lacinulata* (U), *Ecklonia maxima* kelp (K), 16% *Ulva* inclusion formulated feed (F) and a combination of the forementioned diets (U, K, F) rotated on a weekly basis to form a mixed diet (M), on survival rates, feed consumption and feed conversion rates, somatic growth (measured at 23 weeks) and gonad development and quality (measured at 26 weeks) of *P. angulosus* ; and

2. To assess the effects of different temperature treatments including ambient seawater temperature (at Sea Point DFFE marine research aquarium on the Cape southwest coast) and seawater warmed to ca. 20°C, on survival rates, feed consumption and feed conversion rates, somatic growth (measured at 23 weeks) and gonad development and quality (measured at 26 weeks) of *P. angulosus* fed the above diets.

2. Materials and methods

2.1. Ethics statement

Wild *P. angulosus* were collected in intertidal pools and the shallow subtidal on a rocky shore in front of the DFFE Marine Research Aquarium (MRA) in Sea Point, Cape Town (33° 55' 6.492" S, 18° 22' 52.572" E) in May 2023. A fisheries research and development permit to collect, possess, transport, and engage in scientific investigation or practical experiment with the animals was issued successfully in terms of section 83 of the Marine Living Resources Act, 1998 (Act 18 of 1998) permit reference number RES2023/65. All experimental procedures on animals followed the welfare guidelines of the DFFE Aquatic Animal Ethics Committee (AAEC) and Biosecurity programme. This project received ethics approval from the AAEC in the Fisheries Branch of the DFFE under project approval No 20210224_su_03_Macey. Daily biosecurity checks were performed for the duration of the study according to the biosecurity standard of the DFFE.

2.2. Sea urchin collection and acclimatisation

A total of 650 individuals (30 – 50mm in test diameter), which is deemed to be of a sexually mature size (above 30mm) (Greenwood 1980), were collected and immediately transported to plastic tanks in a flow-through system (L x W x D: 91 cm x 58 cm x 60 cm; flow rate ~ 3 L/min) at the MRA. Urchins were starved for three weeks to reduce their gonad weight and ensure that all animals had a similar gonad state prior to the start of the trial. Thereafter, the urchins were stocked in the experimental system (as described in section 2.3.1) and fed small quantities of *Ecklonia maxima* for two weeks while they acclimatised to the new environment.

2.3. Experimental design

2.3.1. Culturing conditions

Urchins were stocked across 32 oyster mesh baskets (n = 19 per basket) (L x W x D: 38 x 28 x 30 cm; mesh size: 6 mm) suspended in plastic tanks (L x W x H: 42 x 36 x 30 cm) with a volume of 40 L. Constant aeration was provided in all tanks using air stones. A flow-through experimental system was utilised for the trials, with standardised flow rates of ~1 L/min (~1.5 tank turnovers/hr). Seawater was pumped from the kelp forest in front of the DFFE MRA. Before entering the experimental systems, seawater passed through a drum filter and then a sand filter prior to entering a sump tank at the highest level of the MRA. For the ambient temperature system, water from the sump was gravity fed from the sump tank into the experimental tanks (n = 16). The urchins were collected in an upwelling region where monthly mean seawater temperatures range from 12.9 – 15.4°C (Smit et al. 2013). The average ambient temperature recorded within the tanks was 15.56 ± 0.07 ºC, slightly warmer than the in-situ temperatures for the region, suggesting that the experimental system itself (pumping, filtration etc.) may have warmed the seawater slightly prior to entering the ambient treatment tanks.

Increasing the culture temperature is hypothesised to increase metabolic rate, resulting in higher rate of feed consumption and growth (somatic and gonadal). For the heated system, water from the main sump at the MRA was gravity fed into two interconnected 2,500 L plastic cylinder tanks where the water was constantly recirculated through a 6.8 kW heat pump before entering the experimental tanks (n = 16) (Figure 7). The temperature (°C) in the ambient and heated experimental tanks was continuously recorded at 30-minute intervals using temperature probes (Star Oddi Starmon Mini temperature recorders). Due to the MRA being in a major upwelling area there are rapid cold spikes in temperature which made it was difficult to maintain high temperatures in the tanks. The heat pump was set to 20 °C but actual temperature within the tanks was ~ 19 °C. Despite this, the heated tank conditions were higher than the average temperature around the Cape Peninsula where densities for this species are highest (~12°C – 17 °C) (Muller et al. 2012, Smit et al. 2013).

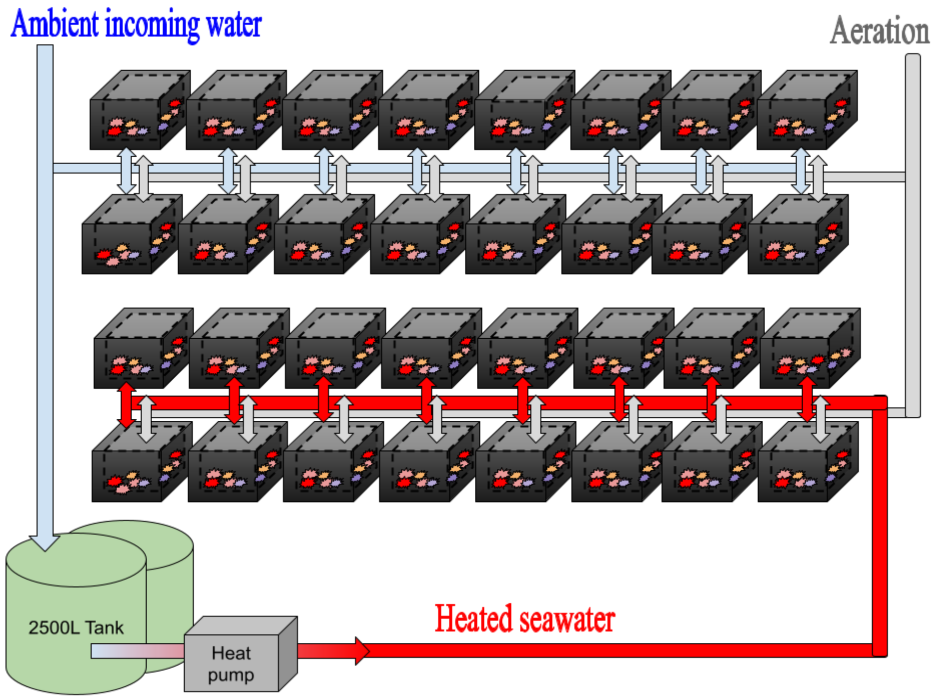


Figure 7. Schematic of the experimental system showing provision and source of water to the temperature treatments (ambient ~ 15°C and heated ~19°C) and aeration to tanks. Mesh baskets suspended inside plastic tanks are indicated as dotted lines. Ambient incoming seawater was gravity fed from a sump tank at the highest level of the MRA after passing through a drum filter and a sand filter.

Effluent water was returned directly to the ocean through the main effluent pipe of the MRA. The internal surfaces of experimental tanks were manually cleaned of their sediments and fouling organisms twice a week, using a siphon and synthetic fibre brush. The experimental system was situated near a window, the tanks received natural sunlight and were left uncovered. To account for variability in sunlight exposure, every 10 days, after cleaning, the baskets within each temperature treatment were rotated between tanks.

2.3.2. Feeds and feeding regimes

Feeds are expected to significantly affect gonad quantity and quality, where feeds with higher protein content (PC) are expected to promote higher gonad yields (Supplementary Table 1) and feeds with higher beta-carotene levels are expected to promote bright (yellow/orange/red) gonad tissue colouration.

Ulva lacinulata and *Ecklonia maxima* were selected as the macroalgae diets in this experiment. *Ulva lacinulata* has a wide range of commercial applications (Bachoo et al. 2023), and along with *Ecklonia maxima* (kelp) is used as a feed on South African abalone farms. *Ulva* has been identified as an effective feeding stimulant for several commercially important sea urchin species, including the local collector sea urchin *T. gratilla* (Cyrus et al. 2015b, Yang et al. 2021, Addis et al. 2023). Protein content levels of *Ulva* (~18%; Supplementary Table 1) are higher in comparison to *Ecklonia maxima* (~11%; Supplementary Table 1) and thus *Ulva*-fed urchins are expected to produce higher gonad yields. Fresh *U. lacinulata* was cultured at the MRA, originally collected from Buffeljags Abalone Farm on the south coast of South Africa (34° 45' 16.2252" S, 19° 36' 52.0308" E) where it was grown in abalone effluent.

Ecklonia maxima (kelp) has a widespread distribution and high densities along the South African west and southwest coasts, making it feasible for regular harvesting. *Ecklonia maxima* is already harvested and used as a feed on South African abalone farms and therefore would be a feasible feed option should the Cape urchin be integrated onto these farms. Kelp has also shown to produce favourable gonad quality for another local urchin species, the collector sea urchin (*T. gratilla*) even though the distribution of *T. gratilla* does not naturally overlap with *E. maxima*. The animals selected for this study were collected from a kelp forest region and therefore assumed to regularly consume kelp debris given the generalist feeding behaviour of the species. Fresh *Ecklonia* regularly washes up onto the shore of Sunset beach, in front of the MRA in the region from which the urchins were collected. Healthy, clean *Ecklonia* fronds were collected, rinsed with seawater and cut into ca. 5 cm segments to increase surface area of feed available to individual urchins and to ensure that all animals had access to feed. To reduce excess mucus effects on water quality in the tanks, old pieces of *Ecklonia* fronds were removed when fresh *Ecklonia* was added.

A formulated feed with 20% (w/w) inclusion of dried *Ulva* has been shown to produce marketable gonad products for *T. gratilla* (Cyrus et al. 2014). The formulated feed used in this study included only 16% (w/w) inclusion of dried *Ulva*. The formulation of the feed was similar to that which was developed for *Tripneustes* by Cyrus (2014) (Supplementary Table 2). Given the 16% inclusion of macroalgae in the formulated feed in the current study it is expected that gonad tissue colouration will be acceptable for market standards and should not be pale in colour. Mixed diets that incorporate natural feeds have been shown to benefit the reproductive performance of *Tripneustes* (Brink-Hull et al. 2022b). Thus, a mixed diet feeding regime which included *E. maxima*, *U. lacinulata* and the formulated feed rotated on a weekly basis was also tested in this study.

Four feeding regimes were tested in quadruplicate for 26 weeks: *Ulva lacinulata* (U), *Ecklonia maxima* kelp (K), a formulated feed containing 16% dry *Ulva* (F) (Cyrus et al. 2014), as well as a rotation of the forementioned diets (U, K, F) on a weekly basis to form a mixed diet (M). Urchins with a similar size range (test diameter) (Table 1) were stocked in each basket across the various treatments to mitigate against growth rate differences due to different sized animals. At the start of the experiment the urchins had a mean ± SEM test diameter of 33.62 ± 0.30 mm and weight of 16.86 ± 0.29 g.

Table 1. Mean ± SEM of initial size of test diameter (mm) and wet weight (g) of *P. angulosus* in each treatment.

Diet	**Temperature**	**Mean ± SEM urchin test diameter (mm)**	**Mean ± SEM whole urchin wet weight (g)**
formulated	ambient	32.91 ± 0.83	16.75 ± 0.77
formulated	warm	35.61 ± 0.73	18.92 ± 0.75
mixed	ambient	32.52 ± 0.85	15.00 ± 0.70
mixed	warm	33.81 ± 0.90	16.91 ± 0.87
U. lacinulata	ambient	33.71 ± 0.88	16.75 ± 0.83
U. lacinulata	warm	33.80 ± 0.81	17.04 ± 0.79
E. maxima	ambient	34.19 ± 0.96	17.83 ± 0.95
E. maxima	warm	32.40 ± 0.72	15.70 ± 0.72

Parechinus angulosus has a wide range of spine/test colour morphs (pink, light purple, dark purple, orange, and red) which are not genetically different to one another (Muller et al. 2012). Where possible, equal ratios of urchins with different test/spine colours were selected for each basket.

To ensure that feed was not a limiting factor for growth and development of urchins, all feeding regimes were administered *ad libitum* three times a week ensuring that feed was constantly available. To avoid overfeeding, the amount of feed added at the start of the experiment was calculated as a percentage of the total body weight within each tank using guidelines for feed provision of *T. gratilla* (U: 8%, F: 1.8%, K: 10%) (BM Macey, pers. comm 2024).

2.3.2.1. Feed stability tests

Formulated feeds may leach organic material by slowly dissolving upon submersion in seawater (Caltagrirone et al 1992) which may impact consumption and digestibility of the feed (Cyrus et al. 2015b, Secci et al. 2021), in addition to adversely impacting water quality. Feed stability tests were therefore conducted within each temperature treatment to assess how much formulated feed is lost (%) due to leaching.

Triplicate samples of 2.54 ± 0.02 g dry weight (mean ± SEM) of formulated feed was added to empty oyster mesh baskets suspended in separate 40 L plastic tanks, supplied with either heated (19ºC) or ambient incoming seawater and fine aeration supplied by air stones (replicating culturing conditions described in section 2.3.1). Formulated feed was weighed to the nearest 0.01 g prior to submersion in seawater and removed after a given time interval (6, 18 or 48 hours). Feed removed from the baskets was dried in a drying oven at 60 °C for 24 hours (to a constant weight) prior to being weighed to the nearest 0.01 g. Simultaneously, triplicate samples of 1.01 ± 0.05 g (mean ± SEM) dry formulated feed was placed in a drying oven at 60 °C for 24 hours prior to being weighed to the nearest 0.01 g to account for any moisture that may be present in the feed prior to submersion.

Feed stability was assessed as percentage dry matter (DM) lost from the feed in seawater at each temperature using the following formula *(Equation 1)*:

$DM\ loss\ (\%) = [(DM_0 - DM_t) \div DM_0] \times 100 - DM\ loss_{control}(\%)$ (Cyrus et al. 2015b)

DM_0 = initial dry weight of feed (g) prior to submersion (g)

DM_t = dry weight of feed after submersion for (t = 6, 18 or 48) hours (g)

$DM\ loss_{control}$ = moisture content (%) in the formulated feed determined by the control test

2.4. Data collection

The urchin data collected for this study included:

- Survival rate (%)
- Somatic growth rate in terms of total urchin wet weight (g), test diameter (mm) and the specific growth rates (SGR; % growth per day)
- Feed consumption rate (g/week)
- Feed conversion ratio (FCR; grams of feed consumed/grams of weight gained)
- Gonad quantity (g) and gonad somatic index (GSI (%) = gonad weight (g) /animal weight (g) * 100)
- Gonad quality (gonad colour) based on quantitative assessment (CIE L*a*b*)
- Gonad development (gonad maturity) based on histological analysis.

2.4.1. Survival rate (%)

Daily biosecurity checks were performed, during which time any mortalities were recorded and removed from the tanks. The tank number was noted to establish the mean ± SEM survival rate across the various treatments (%).

2.4.2. Somatic growth

Sea urchin total wet weight (g) and test diameter (cm) was measured at six measurement timepoints (T0: initial, T1: 4 weeks, T2: 8 weeks, T3: 13 weeks, T4: 18 weeks, T5: 23 weeks) to assess somatic growth across dietary and temperature treatments over the course of the study. At every timepoint, all sea urchins were individually weighed to the nearest 0,01 g using an electronic scale to determine mean ± SEM weight and a standardised photograph was taken per tank to determine mean ± SEM sea urchin test diameters (cm) across treatments. The standardised photographs were taken using an iPhone 8 according to the methodology provided by De Vos et al. (2023) and processed using their open-source “Urchin Vision” software.

To account for differences in initial diameters and weights, specific growth rate (SGR) was calculated to measure growth over time. Sea urchin weight (g) and diameter (cm) was averaged per tank and used to calculate the specific growth rate (SGR). The SGR (% growth/day) was calculated for each treatment across five intervals (between each respective timepoint) using mean weight (g) and size (cm) measurements per timepoint per tank across treatment using the following formula *(Equation 2)*:

$$SGR = 100 * \frac{(ln\ (\frac{m_f}{m_i}))}{\Delta}$$

Where m_f = measurement final mass (g) or diameter (cm); m_i = measurement initial (g or cm); Δ = time (days) between final and initial measurements.

The intervals for the five SGRs calculated were as follows:

1. week 0 – week 4 (t = 29 days)
2. week 4 – week 8 (t = 29 days)
3. week 8 – week 13 (t = 32 days)
4. week 13 – week 18 (t = 36 days)
5. week 18 – week 23 (t = 36 days)

2.4.3. Feed consumption and feed conversion ratio (FCR)

The total feed consumed per tank per week was calculated as the difference in mass (g) between the feed introduced and the residual feed removed from the tank. Dry matter loss of formulated feed due to leaching (see section 2.3.2.1) was considered when feed consumption values were calculated for each temperature treatment.

The feed consumption was then divided by the number of animals in the tank to calculate the average feed (dry weight in grams) consumed per animal per week using the following formula *(Equation 3):*

$$Feed\ consumed\ per\ urchin\ (g)\ =\frac{Feed\ added\ (g) - Residual\ feed\ removed\ (g)\ per\ tank}{Number\ of\ urchins\ in\ the\ tank}\times(1-leaching\ (\%))$$

Where $leaching$ (%) = 0 for *U. lacinulata* and *E. maxima*

To allow the sea urchins to adapt to the treatments before feed consumption was measured, feed consumption measurements were only recorded from week 8 onwards. Feed consumed per urchin per week (g) was measured at every somatic growth measurement timepoint from week 8 onwards (every 5 weeks as described in section 3.6.3). Feed consumption per week (g) was extrapolated (multiplied by 5) to account for feed consumed in the weeks between measurement timepoints and added together to determine average feed consumed per urchin over the entire experimental period (g).

The macroalgae (*U. lacinulata* and *E. maxima*) used as feeds in this study remained intact when residual feed material was removed to assess feed consumption and therefore, no degradation and weight reduction was considered for these feeds. The seaweeds were spun using a salad spinner for 30 rotations (Brand 2023) to remove excess moisture before they were weighed. The formulated feed was dried to a constant weight (in an oven at 60ºC for 48 hours) before being weighed.

Feed consumed (g) was divided by the total wet weight gain (g) over the experimental period to determine the feed conversion ratio (FCR), which was calculated as an average per tank. *(Equation 4)*:

$$FCR=\frac{Feed\ consumed\ per\ urchin\ (g)}{Urchin\ wet\ weight\ gain\ (g)}$$

2.4.4. Gonad quantity and quality

Gonad quantity was assessed by measuring whole urchin wet weight (g) and gonad wet weight (g) and calculating gonad somatic index (GSI; %) across dietary and temperature treatments. Gonads cannot be measured without sacrificing the urchins thus, to ensure that sample sizes were not reduced too drastically during early stages of the experiment only four sea urchins per treatment (i.e., one per tank) were dissected in week 9 and 19 for this assessment, whereas twelve sea urchins per treatment (i.e., three per tank) were dissected at the final measurement timepoint in week 26 of the experiment. Twelve urchins were also dissected from the kelp diet treatment in week 9, when kelp was removed as a dietary treatment due to poor growth and high mortality rates (explanation provided in the Results section 3.3).

Prior to dissection, the whole urchin wet weight (g) was recorded to the nearest 0.01g after which each urchin was carefully dissected in half, avoiding damage to the gonads. All other visceral tissues attached to the gonad were carefully removed using dissection scissors and tweezers before the five gonad segments were removed from the test using a spatula and weighed to the nearest 0.01g to quantify the total gonad wet weight (g) and GSI (%) was calculated using the following formula *(Equation 5)*:

$$GSI\ (\%) = \frac{gonad\ wet\ weight\ (g)}{whole\ urchin\ wet\ weight\ (g)} \times 100$$

Qualitative aspects of sea urchin gonad tissue such as colour and taste are often given an eye-rated or taste-rated assessment in relation to market desirability, however this is subjective. Quantitative analyses of gonad colour are widely used in echinoculture research using a three-dimensional colour method by the International Commission on Illumination (CIE) which quantifies intensity/lightness (L*), hue/redness (a*), and chroma/yellowness (b*) respectively (Figure 8) (Robinson et al. 2002, Symonds et al. 2009, Cyrus et al. 2014, Onomu et al. 2020, Lourenço et al. 2022, Suckling et al. 2022). Using a hand-held fibre-optic spectrophotometer (Lovibond® LC 100 spectrocolorimeter), three replicate measurements of CIE L*, a* and b* were recorded for one gonad from each dissected urchin and averaged to account for any variation across individual readings.

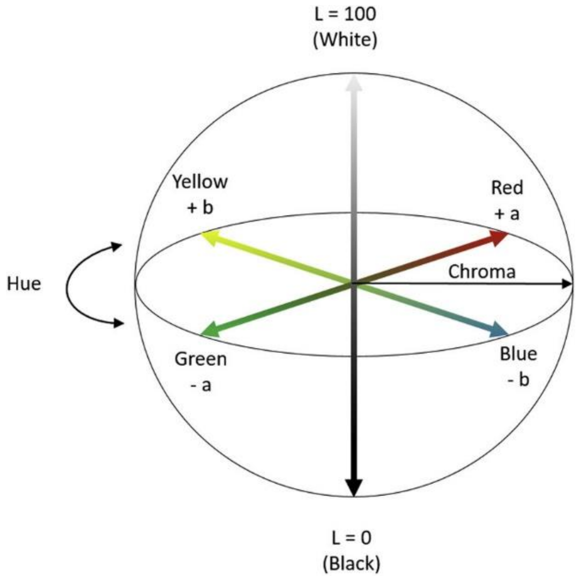


Figure 8. A visual representation of the CIE Lab colour space (Ly et al. 2020)

One gonad per urchin was processed to assess gonad development (gametogenic state) through histological analysis. Each gonad was fixed in Davidson's fixative (per litre: 300 mL 95% ethyl alcohol, 200 mL 100% formalin, 100 mL glycerol, 100 mL glacial acetic acid and 300 mL distilled water) for a period of 48 hours. Thereafter, the samples were transferred to 70% ethanol for storage prior to paraffin histology (Cyrus et al. 2015a, Brink-Hull et al. 2022b). Gonad maturity was analysed according to the method described by Cyrus et al. (2015). Gonads were categorised into one of six different maturity stages, namely (1) recovery, (2) growing, (3) premature, (4) mature, (5) partly spawned, and (6) spent (Figure 6). Gonads having little or no gametogenic activity, considered as high-quality, are gonads in the growing or premature stages (Onomu et al., 2020).

2.5. Statistical Analyses

Data analyses were performed, and plots were drawn using the ggplot2 package (Wickham 2017) in R statistical software (R Core Team 2023). This study involved multiple datasets across four dietary treatments and two temperature conditions: temperature (ºC), survival rate (%), whole urchin wet weight (g), urchin test diameter (cm converted to mm), somatic growth rate (SGR) (%) (in terms of weight and size), feed consumption rate (g/week), feed conversion ratio (FCR), gonad weight (g), gonad somatic index (GSI; %), gonad colour (CIE

L*a*b values) and gonad development (maturity). An F-test was used to assess variability in the temperature (°C) dataset. To test if gonad maturity was independent of diet and temperature the Fisher exact test (Sprent 2011) was used, followed by a Kruskal-Wallis test (Kruskal and Wallis 1952) to assess differences between dietary groups. All other datasets were analysed according to the methodology below.

Datasets were tested for normality (Shapiro-Wilk) (Shapiro and Wilk 1965) and homogeneity of variance (Levene's test) using the *car* R package (Fox and Weisberg 2019; Levene 1960). If these assumptions were met, a two-way analysis of variance (ANOVA; Fisher 1970) was conducted to assess dietary treatment effects, temperature treatment effects and differences within each treatment over time. Where statistically significant dietary treatment effects or differences within a treatment over time were observed ($p < 0.05$), a post-hoc Tukey (Keselman and Rogan 1977) pairwise comparison was conducted to identify significant group differences. Two sample t-tests were conducted to assess differences across temperature treatments within each dietary treatment group.

If data failed to meet the assumptions of normality following either a log or square root transformation[1], a nonparametric Kruskal Wallis test was conducted for dietary treatment effects (within each temperature treatment) and differences within each treatment over time. Mann-Whitney U tests (MacFarland and Yates 2016) were conducted (within each dietary treatment) to assess temperature treatment effects (using the *coin* R package; Hothorn et al., 2006). Where significant results were found for dietary treatment effects or differences within a treatment over time ($p < 0.05$), a Dunn's post-hoc comparison was conducted to determine group differences (using the *dunn.test* R package; ; Dinno 2015). To account for multiple testing, a Bonferroni correction was applied to reduce the type I error rate.

If data had a normal distribution but failed to meet the assumption of homogeneity, a Welch ANOVA (Welch 1951) was conducted for dietary treatment effects (within each temperature treatment) and differences within each treatment over time. Welch two sample t-tests (within each dietary treatment) were conducted to assess temperature treatment effects. Where statistically significant dietary treatment effects or differences within a

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[1] Where negative values were present, the data were log or square root transformed as follows:
log10(max(x+1) - x) or *sqrt(max(x+1) - x)*

treatment over time were observed ($p < 0.05$), Games-Howell (Games and Howell 1976) post-hoc comparisons were conducted to determine group differences (using the *rstatix* R package; Kassambara, 2021).

Lastly, the *ggplot2* package was used to perform a principal component analysis (PCA) of gonad tissue colour, to assess differences in gonad tissue colour profiles across diets after 26 weeks, temperature treatment and sex were not considered in this analysis.

3. Results

In this study, four feeding regimes (*E. maxima* (kelp) = K, *U. lacinulata* = U, formulated = F, mixed = M) and two temperature treatments (ambient = A, warm = W) were assessed for growing the Cape urchin for 26 weeks under aquaculture conditions. Treatments are therefore abbreviated based on the diet followed by their temperature throughout the results of this study e.g., MA refers to the mixed dietary treatment and ambient temperature treatment.

3.1. Feed stability

The moisture content of the formulated feed was 6.47 ± 0.12 % (mean ± SEM) (see section 2.3.2.1). The average dry matter loss due to leaching, after taking moisture content of the feed into account, was significantly higher in the warm temperature than the ambient temperature treatment (warm: 25.74 ± 0.98 %; ambient: 16.10 ± 0.26 %) (Mann-Whitney, $p < 0.05$). The formulated feed was found to be unstable, having greater than 20% dry matter loss in the warm temperature treatment, after 48 hours. Therefore, every 48 hours tanks were supplied with new formulated feed and any residual feed was removed from the tanks.

3.2. Temperature

Temperature was continuously monitored within each temperature treatment. The mean ± SEM temperature (ºC) over the entire experimental period was 15.56 ± 0.07 ºC and 18.94 ± 0.03 ºC for the ambient and warm temperature treatments, respectively. Temperatures ranged from 12.33 – 18.62 ºC and 17.06 – 19.92 ºC for the ambient and warm temperature treatments, respectively. High variability in the ambient temperature treatment data was recorded from September to December, which correlates with the upwelling season (spring/early summer) in the Southern Benguela (Figure 9). Temperature records for the ambient treatment were significantly more variable in spring and early summer (September – December) than in winter (June – August) (F – test, $p < 0.001$). Somatic growth measurements were evenly split outside (week 0, 4 & 8) and within (week 13, 18 & 23) the upwelling season. The first gonad growth measurements (August; week 9) were in winter, the second measurements occurred in spring (October; week 19) and the final measurements in summer (December; week 23).

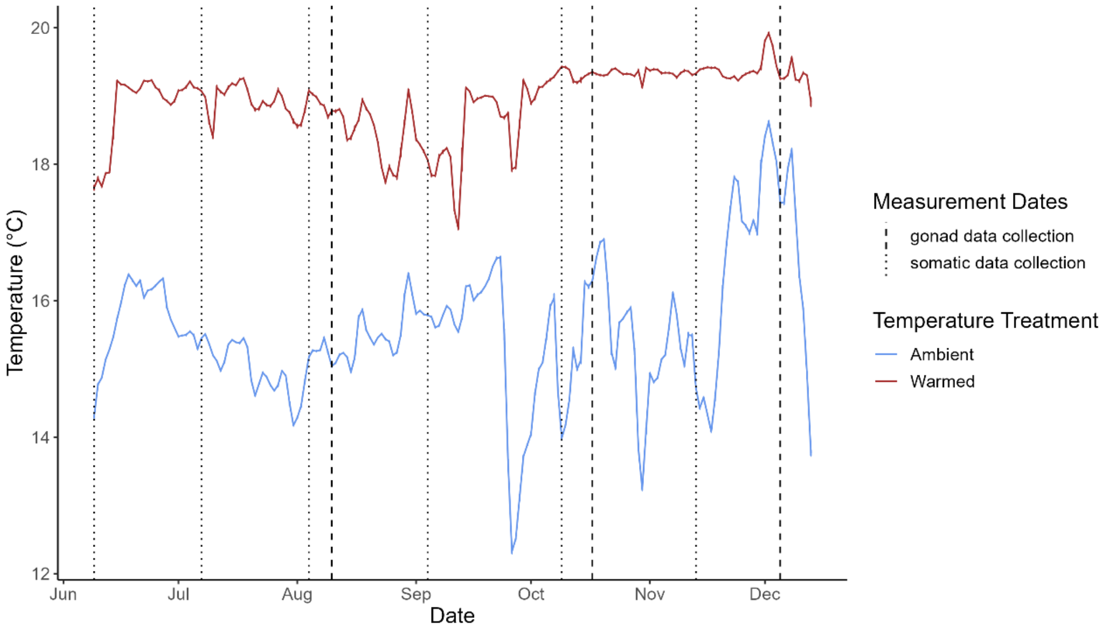

Figure 9. Temperature (ºC) recorded for across the ambient and warm temperature treatments over a 6-month period.

3.3. Survival

Diet had a significant effect on survival rate (%) across both temperature treatments (Kruskal-Wallis, $p < 0.05$) within the first nine weeks of the experiment (Figure 10). Kelp-fed urchins had the lowest survival rates (KA $_{\text{week 9}}$: 67.11 ± 6.58%; KW $_{\text{week 9}}$: 65.79 ± 6.26%), significantly lower than mixed diet-fed urchins (MA $_{\text{week 9}}$: 97.37 ± 2.63 %; MW $_{\text{week 9}}$: 100.00 ± 0.00%) which had the highest survival rate in this study (Dunn Bonferroni-corrected test, $p < 0.05$). Many kelp-fed urchins showed severe spine loss and were removed from the tanks for disease and water quality concerns (Supplementary Figure 1). Due to the consistently poor health during the early stages of the experiment and concerns for the health of the animals, the kelp dietary treatment was suspended in week 9. Urchins within this treatment group were ethically euthanized as per DFFE biosecurity regulation and kelp was removed from the mixed dietary treatment feeding regime for the remainder of the experimental period. The mixed diet regime changed to *U. lacinulata* and a formulated feed containing 16% *Ulva* rotated on a weekly basis from week 10 onwards.

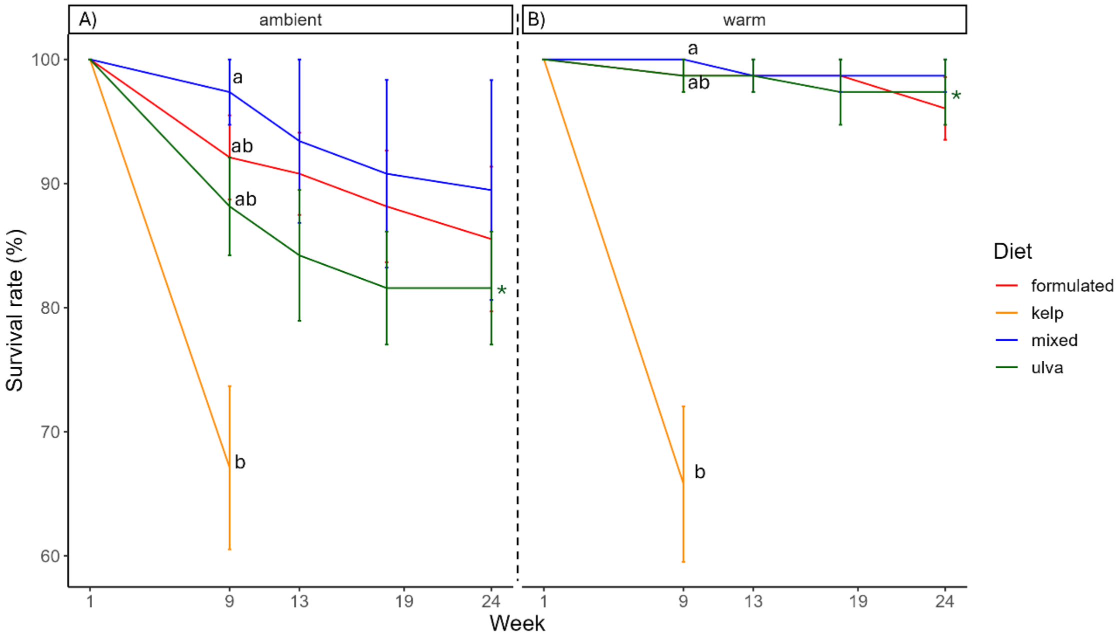

Figure 10. Mean (± SEM) survival rate (%) for the Cape sea urchin *P. angulosus* fed four different dietary treatments (formulated feed with 16% dried *U. lacinulata*, *E. maxima* kelp, fresh *U. lacinulata*, and a combination of the forementioned diets to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

Ulva-fed urchins had significantly lower survival rates when maintained at ambient temperatures than warm temperatures (UA: 81.58 ± 4.56 %; UW: 97.37 ± 2.63 %) (Mann-Whitney, $p < 0.05$). Overall, urchins maintained at warm temperatures had a significantly higher survival rate on average (97.37 ± 1.21 %) than urchins maintained at ambient temperatures (85.53 ± 3.61%) (Mann-Whitney, $p < 0.05$). Tanks within the ambient temperature treatment received higher sediment loads from seawater than tanks within the heated treatment as this seawater did not flow through a sump first as in the warm water temperature treatment group, which was noticeable during weekly biweekly tank surface cleaning.

3.4. Somatic growth

3.4.1. Whole urchin size

3.4.1.1. Urchin test diameter (mm)

The urchins grew from (mean ± SEM) 33.62 ± 0.30 mm to 38.75 ± 0.38 mm within a 23-week experimental period. The initial (week 0) test diameters (TD, mm) of urchins selected for the warm temperature treatment was significantly larger than urchins selected for the ambient temperature treatment within the formulated dietary treatment group ($\mathrm{FA}_{\mathrm{initial}}$: 32.91 ± 0.83 mm; $\mathrm{FW}_{\mathrm{initial}}$: 35.61 ± 0.73 mm) (T-test, $p < 0.05$). Within the warm temperature treatment, urchins selected for the formulated feed treatment were significantly larger than urchins selected for the kelp diet ($\mathrm{FW}_{\mathrm{initial}}$: 35.61 ± 0.73 mm; $\mathrm{KW}_{\mathrm{initial}}$: 32.40 ± 0.72 mm) (Tukey, $p < 0.05$). After 8 weeks, TD of kelp-fed urchins remained significantly smaller than urchins fed formulated feed ($\mathrm{FW}_{\mathrm{week\ 8}}$: 36.98 ± 0.78 mm; $\mathrm{KW}_{\mathrm{week\ 8}}$: 32.98 ± 0.86 mm) (Tukey, $p < 0.05$). After the suspension of the kelp dietary treatment in week 9, no significant differences between dietary or temperature treatments were found at week 13, 18 and 23. (ANOVA, $p > 0.05$ (Figure 11).

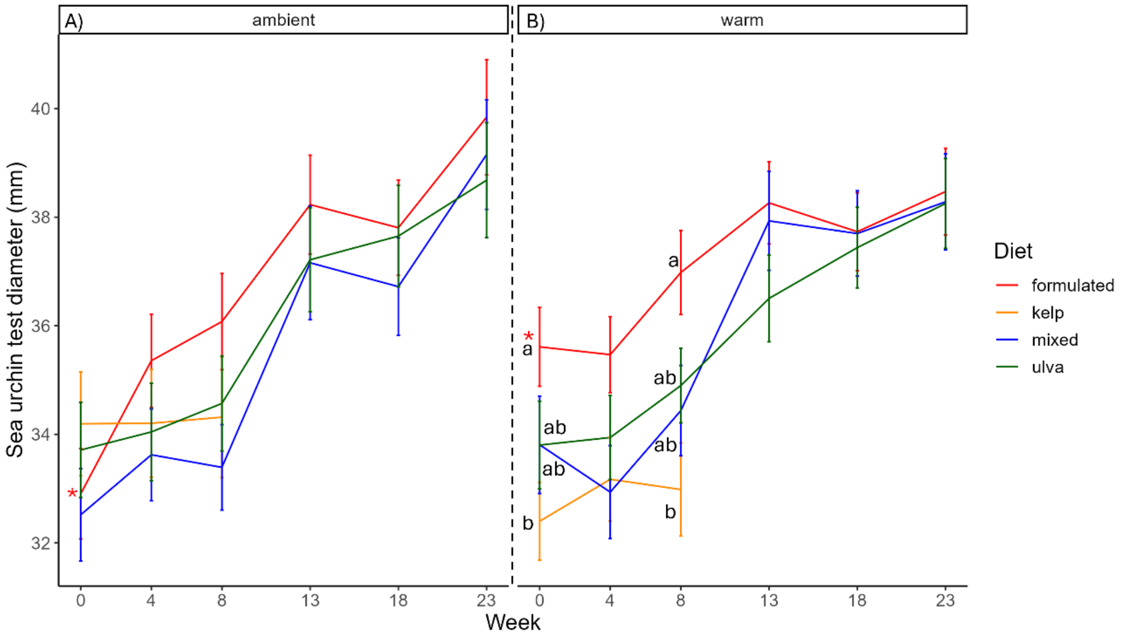

Figure 11. Mean (± SEM) test diameter (mm) for the Cape sea urchin *P. angulosus* fed four different dietary treatments (formulated feed with 16% dried *U. lacinulata*, *E. maxima* kelp, fresh *U. lacinulata*, and a combination of the forementioned diets to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

3.4.1.2.Specific growth rate (SGR; % growth/day) of urchin test diameter (mm)

To account for differences in starting test diameters, specific growth rate (SGR) was calculated to measure test diameter growth over time.

Diet had no effect on the SGR of test diameters of Cape urchins in this study. However, test diameters of mixed diet-fed urchins maintained at ambient temperature grew significantly faster (% growth/day) than urchins in the same dietary treatment maintained at warm temperature between week 18 and 23 ($MA_{\text{week 23}}$: 0.18 ± 0.02 % growth/day; $MW_{\text{week 23}}$: 0.04 ± 0.03 % growth/day) (Two sample t-test, $p < 0.05$) (Figure 12). SGR oscillated over time across all diets, with sharp declines in SGR between week 13 and 18. The SGR of sea urchin test diameter overall, considering the entire 23-week experimental period, was ca. 0.1 % growth/day across all treatments.

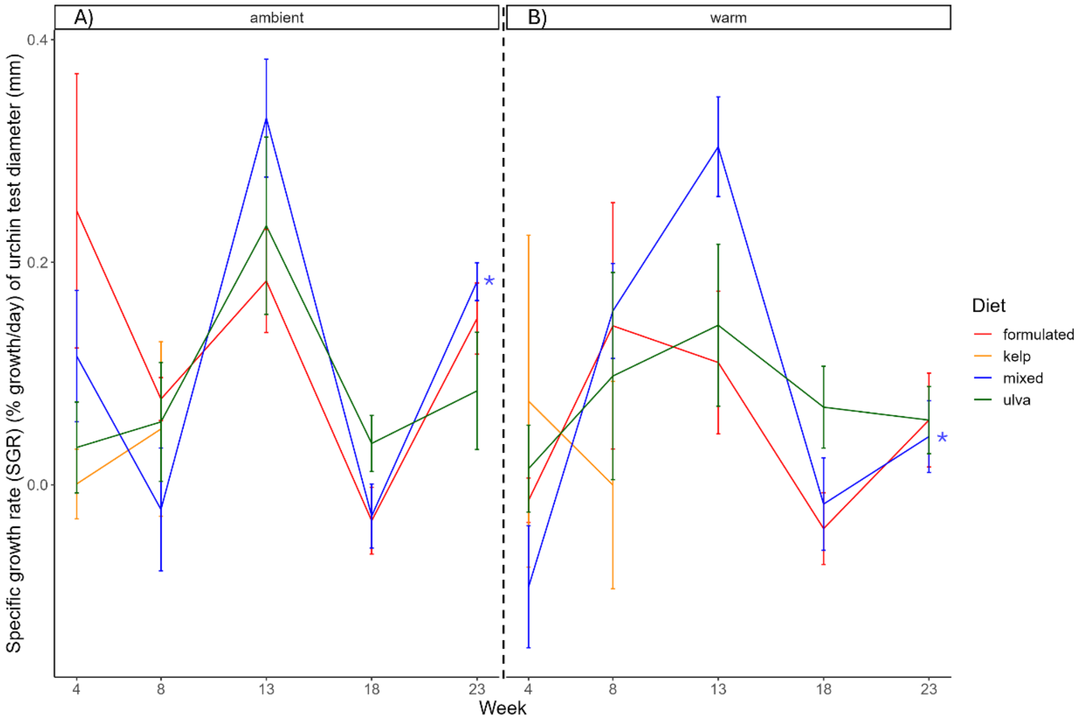

Figure 12. Mean (± SEM) specific growth rate (SGR) (% growth/day) of test diameter (mm) for the Cape sea urchin *P. angulosus* fed four different dietary treatments (formulated feed with 16% dried *U. lacinulata*, *E. maxima* kelp, fresh *U. lacinulata*, and a combination of the forementioned diets to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint. No statistically significant differences between diets were found at any specific timepoint and temperature.

3.4.1.3.Test diameter increase (mm)

Diet had no effect on the overall test diameter increase (mm) of Cape urchins after 23 weeks in this study (ANOVA, $p > 0.05$). Formulated feed-fed urchins had a significantly greater increase in overall test diameter when maintained at ambient temperatures than at warm temperatures (FA: 7.07 ± 0.76 mm; FW: 2.97 ± 1.02 mm) (Two sample t-test, $p < 0.05$). Similarly, Cape urchins fed other diets had a greater increase in overall test diameter when maintained at ambient temperatures than warm temperatures (MA: 6.73 ± 0.90 mm; MW: 4.50 ± 0.46 mm) (UA*:* 5.28 ± 1.21 mm; UW: 4.46 ± 0.73 mm) (Figure 13).

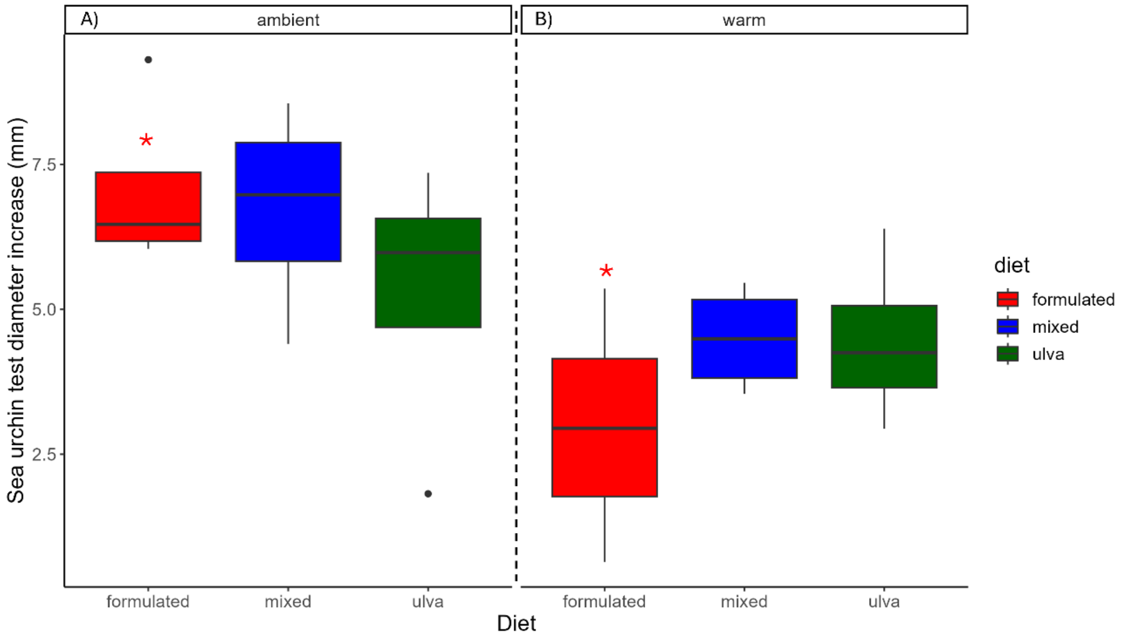

Figure 13. Boxplot of test diameter increase (mm) over 23 weeks for the Cape sea urchin, *P. angulosus*, fed four different dietary treatments (formulated feed supplemented with 16% *Ulva*, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. No statistically significant differences were found between diets within temperature treatments. Stars indicate statistically significant differences between temperatures for a specific diet.

3.4.2. Whole urchin weight

3.4.2.1.Whole urchin wet weight (g)

The urchins grew from (mean ± SEM) 16.86 ± 0.29 g to 22.00 ± 0.37 g within a 23-week experimental period. The initial whole urchin wet weights (WW, g) of urchins selected for the formulated feed treatment were significantly larger than urchins selected for the kelp diet within the warm temperature treatment (FW$_{\text{week 0}}$: 18.92 ± 0.75 g; KW$_{\text{week 0}}$:15.70 ± 0.72 g) (Tukey, $p < 0.05$). Kelp-fed urchins consistently exhibited significantly lower whole urchin WW values to formulated feed-fed urchins (KW$_{\text{week 4}}$: 15.25 ± 0.73 g; KW$_{\text{week 8}}$: 16.11 ± 0.85 g) (FW$_{\text{week 4}}$: 20.04 ± 0.77 g ; FW$_{\text{week 8}}$: 21.39 ± 0.76 g) (Dunn Bonferroni-corrected $p < 0.05$) (Figure 14). After the suspension of the kelp dietary treatment in week 9, no dietary or temperature treatment effects were observed for whole urchin wet weight (g) of Cape urchins at week 13, 18 and 23 of this study.

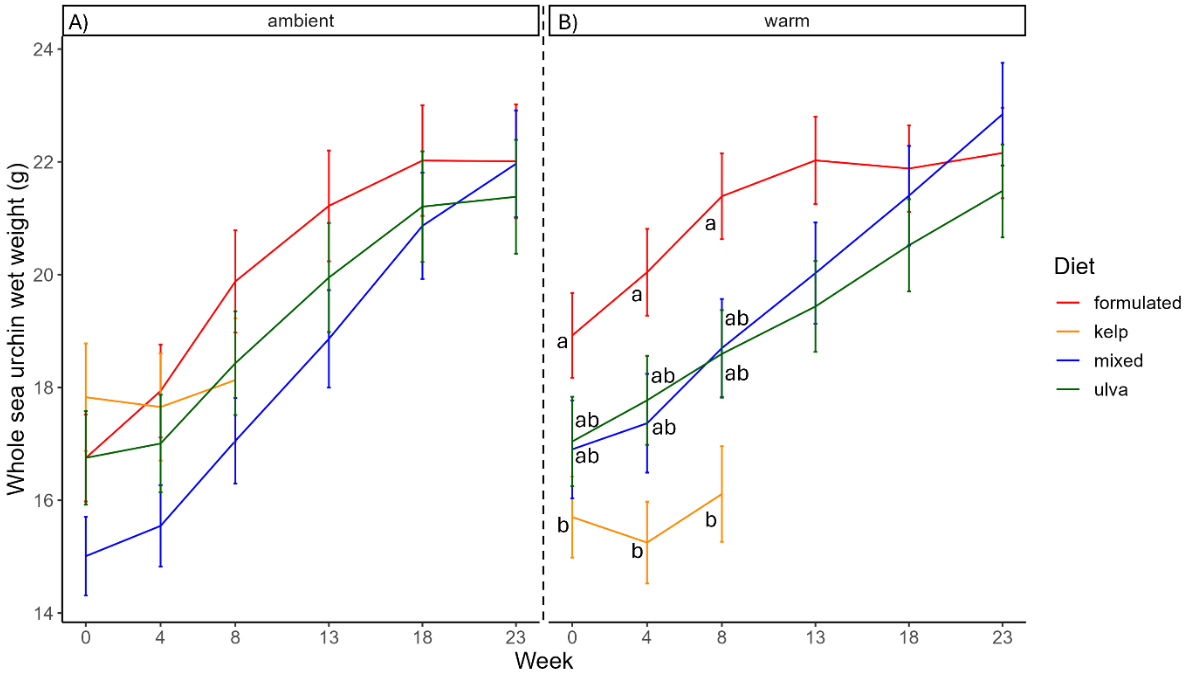

Figure 14. Mean (± SEM) of whole urchin wet weight (g) for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. No statistically significant differences between temperatures were found for any specific diet and timepoint.

3.4.2.2.Specific growth rate (SGR; % growth.day^{-1}) of whole urchin wet weight (g)

To account for differences in starting whole urchin wet weights, SGR of whole urchin wet weight was assessed.

In the first four weeks, urchins fed formulated feed gained weight significantly faster than urchins fed other diets when maintained at ambient temperature (FA$_{\text{week 4}}$: 0.24 ± 0.03 % growth.day^{-1}) (Tukey, $p < 0.05$). Across both temperature treatments, kelp-fed urchins exhibited the lowest SGR of whole-body wet weight (g), losing weight during the first four weeks of the experiment (KA$_{\text{week 4}}$: -0.03 ± 0.03 % growth.day^{-1}; KW$_{\text{week 4}}$: -0.10 ± 0.04 % growth.day^{-1}). Within the warm temperature treatment, the SGR of kelp-fed urchins was significantly lower than all other dietary treatments (MW$_{\text{week 4}}$: 0.10 ± 0.02 % growth.day^{-1}; UW$_{\text{week 4}}$: 0.14 ± 0.03 % growth.day^{-1}; FW$_{\text{week 4}}$: 0.20 ± 0.02 % growth.day^{-1}) (Tukey, $p < 0.01$). Within the ambient temperature treatment, urchins fed fresh *U. lacinulata* had a similarly low SGR for whole urchin wet weight (UA$_{\text{week 4}}$: 0.05 ± 0.02 % growth.day^{-1}) (Figure 15).

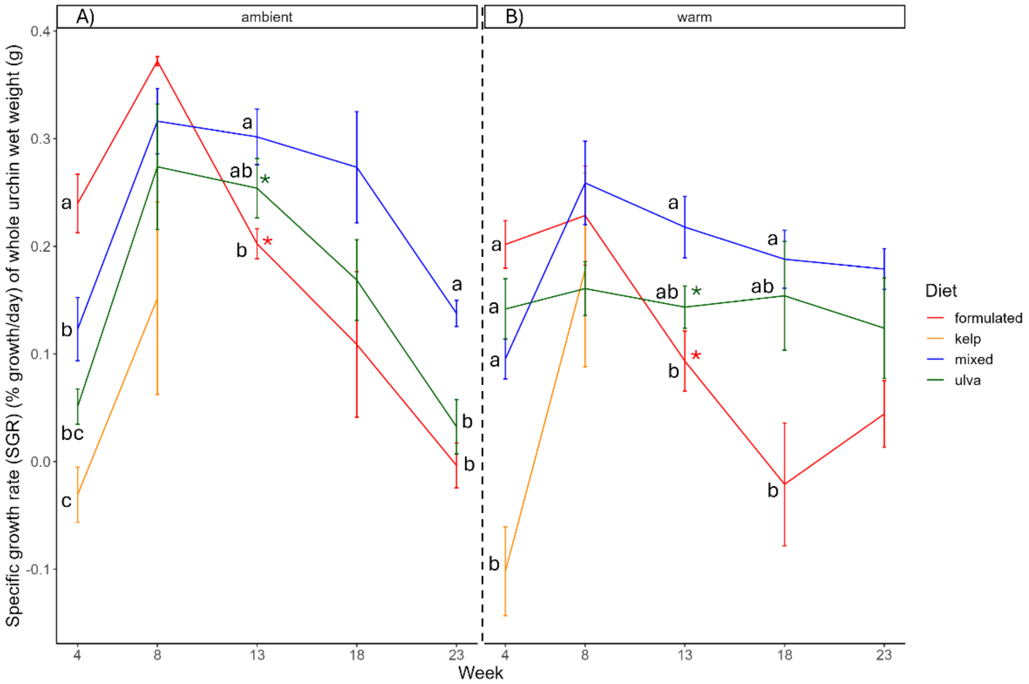

Figure 15. Mean (± SEM) specific growth rate (SGR) (% growth/day) of whole urchin wet weight (g) for the Cape sea urchin *P. angulosus* fed four different dietary treatments (formulated feed with 16% dried *U. lacinulata*, *E. maxima* kelp, fresh *U. lacinulata*, and a combination of the forementioned diets to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

Kelp-fed urchins maintained at warm temperature showed a significant improvement in whole body wet weight (g) SGR between week 4 and 8 (KW$_{\text{week 8}}$: 0.18 ± 0.09 % growth.day^{-1}) (Tukey, $p < 0.05$). However, the kelp dietary treatment was suspended in week 9. As a result, kelp was removed from the mixed diet feeding regime in week 10. This had no immediate effect on the SGR of whole urchin wet weight (g) for mixed diet-fed urchins (MA$_{\text{week 8}}$: 0.32 ± 0.03 % growth.day^{-1}; MA$_{\text{week 13}}$: 0.30 ± 0.03 % growth.day^{-1}; MW$_{\text{week 8}}$: 0.26 ± 0.04 %

growth.day^{-1}; MW $_{\text{week 13}}$: 0.22 ± 0.03 % growth.day^{-1}) (Tukey, $p > 0.05$). However, due to consistent SGR, mixed diet-fed urchins gained weight significantly faster than formulated feed-fed urchins (MA $_{\text{week 13}}$: 0.30 ± 0.03 % growth.day^{-1}; FA $_{\text{week 13}}$: 0.20 ± 0.01 % growth.day^{-1}) (MW $_{\text{week 13}}$: 0.22 ± 0.03 % growth.day^{-1}; FW $_{\text{week 13}}$: 0.09 ± 0.03 % growth.day^{-1}) (Tukey, $p < 0.05$). The SGR of formulated feed-fed urchins declined significantly following a peak in week 8 (FA $_{\text{week 8}}$: 0.37 ± 0.00; FW $_{\text{week 8}}$: 0.23 ± 0.05), such that no growth was observed by week 23 (FA $_{\text{week 23}}$: 0.00 ± 0.02 % growth.day^{-1}; FW $_{\text{week 23}}$: 0.04 ± 0.03 % growth.day^{-1}) (Tukey, $p < 0.05$). Similarly, *Ulva*-fed urchins exhibited significant declines in SGR, with no growth observed by week 23, when maintained at ambient temperature (UA $_{\text{week 8}}$: 0.27 ± 0.06 % growth.day^{-1}; UA $_{\text{week 13}}$: 0.25 ± 0.03 % growth.day^{-1}; UA $_{\text{week 18}}$: 0.17 ± 0.04 % growth.day^{-1}; UA $_{\text{week 23}}$: 0.03 ± 0.03 % growth.day^{-1}) (Tukey, $p < 0.01$). The SGR of *Ulva*-fed urchins was consistent when maintained at warm temperatures although significantly lower than when maintained at ambient temperature (UW $_{\text{week 13}}$: 0.14 ± 0.02 % growth.day^{-1}; UA $_{\text{week 13}}$: 0.25 ± 0.03 % growth.day^{-1}) (Two sample t-test, $p < 0.05$).

3.4.2.3. Whole sea urchin weight gain (g)

Mixed diet-fed urchins exhibited the highest overall weight gain (g) (MA: 6.78 ± 0.61 g; MW: 5.96 ± 0.47 g) (Figure 16). When urchins were maintained at ambient temperatures, mixed diet-fed urchins gained significantly more weight than urchins fed fresh *U. lacinulata* (MA: 6.78 ± 0.61 g; UA: 4.66 ±0.44 g) (Tukey, $p < 0.05$). When urchins were maintained at warm temperatures, mixed diet-fed urchins gained significantly more weight than urchins fed formulated feed (MW: 5.96 ± 0.47 g; FW: 3.34 ± 0.36 g) (Tukey, $p < 0.05$). Formulated feed-fed urchins exhibited significantly higher overall weight gain (g) when maintained at ambient temperature than at warm temperature (FA: 5.34 ± 0.41 g; FW: 3.34 ± 0.36 g) (Two sample t-test, $p < 0.05$)

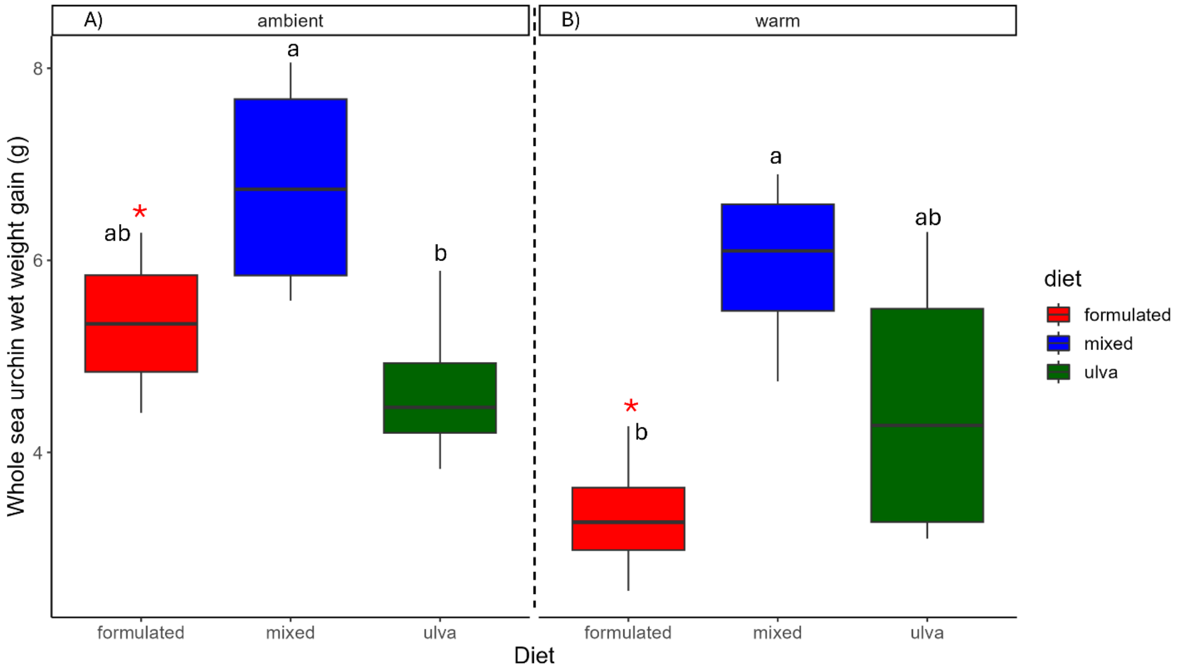

Figure 16. Boxplots of whole urchin wet weight gain (g) over 23 weeks for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. Different letters indicate statistically significant differences between diets at a specific temperature. Stars indicate statistically significant differences between temperatures for a specific diet.

3.5. Feed consumption rate and feed conversion ratio (FCR)

3.5.1. Feed consumption rate (g/week per urchin)

Urchins fed the fresh *U. lacinulata* diet and maintained at warm temperatures had significantly higher consumption rates than urchins maintained at ambient temperatures at week 13 ($UW_{\text{week } 13}$: 3.40 ± 0.18 g/week; $UA_{\text{week } 13}$: 2.17 ± 0.05 g/week) and 23 ($UW_{\text{week } 23}$: 2.57 ± 0.12 g/week; $UA_{\text{week } 23}$: 1.03 ± 0.22 g/week) of the experiment (Mann-Whitney, $p < 0.05$). Similar results were observed for urchins fed the mixed diet feeding regime, which consisted of a rotation of formulated feed and *U. lacinulata* every alternate week. When the urchins within the mixed diet feeding regime were fed fresh *U. lacinulata* during week 13 and 23, feed consumption rate (g/week) was significantly higher when urchins were maintained at warm temperature ($MW_{\text{week } 13}$: 2.75 ± 0.20 g/week; $MW_{\text{week } 23}$: 1.97 ± 0.22 g/week) than ambient temperature ($MA_{\text{week } 13}$: 1.42 ± 0.07 g/week; $MA_{\text{week } 23}$: 1.17 ± 0.12 g/week) (Mann-Whitney, $p < 0.05$). Temperature had no effect on feed consumption rate for urchins fed the formulated feed.

The Cape sea urchin consistently consumed *Ulva* at significantly higher rates than formulated feed (UA $_{\text{week 23}}$: 1.03 ± 0.22 g/week; FA $_{\text{week 23}}$: 0.37 ± 0.02 g/week) (UW $_{\text{week 23}}$: 2.57 ± 0.12 g/week; FW $_{\text{week 23}}$: 0.37 ± 0.01 g/week) (Dunn Bonferroni-corrected, $p < 0.05$) (Figure 17). Urchins consumed kelp (KA $_{\text{week 8}}$: 2.05 ± 0.26 g/week; KW $_{\text{week 8}}$: 2.38 ± 0.17 g/week) at a similar rate to *U. lacinulata* (UA $_{\text{week 8}}$: 2.30 ± 0.22 g/week; UW $_{\text{week 8}}$: 3.13 ± 0.12 g/week) in week 8, prior to the suspension of the kelp dietary treatment (Dunn Bonferroni-corrected, $p > 0.05$).

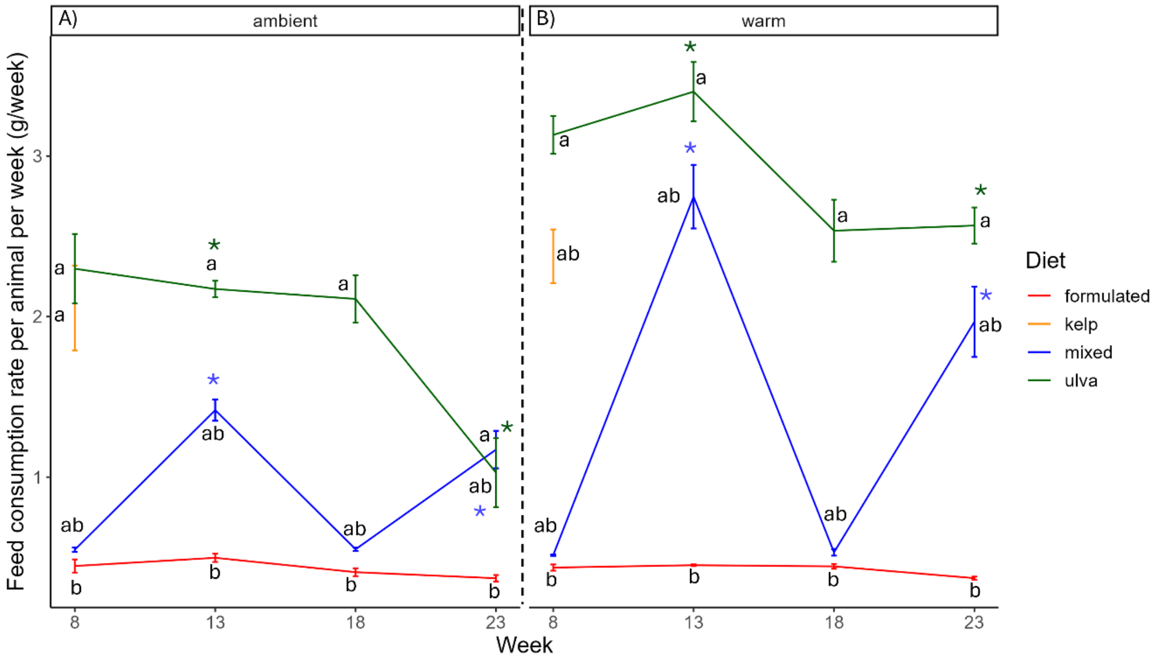

Figure 17. Mean (± SEM) of feed consumption rate per animal (g/week) for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

To assess whether *U. lacinulata* acts as a feeding stimulant for this species, feed consumption rates of formulated feed for urchins in the mixed and formulated diet feeding regimes were compared. When the urchins in the mixed diet feeding regime were fed formulated feed, in week 8 (MA $_{\text{week 8}}$: 0.55 ± 0.01 g/week; MW $_{\text{week 8}}$: 0.51 ± 0.00 g/week) and 18 (MA $_{\text{week 18}}$: 0.55 ± 0.01 g/week; MW $_{\text{week 18}}$: 0.53 ± 0.02 g/week), they consumed formulated feed at a significantly higher rate than urchins which were fed formulated feed exclusively (FA $_{\text{week}}$

$_8$: 0.45 ± 0.04 g/week; FW $_{\text{week 8}}$: 0.44 ± 0.02 g/week; FA $_{\text{week 18}}$: 0.41 ± 0.02 g/week; FW $_{\text{week 18}}$: 0.44 ± 0.01 g/week) across both temperature treatments (Mann-Whitney $_{\text{ambient}}$, $p < 0.01$; Two sample t-test $_{\text{warm}}$, $p < 0.001$).

3.5.2. Feed conversion ratio (FCR; feed consumed (g)/weight gained (g))

Urchins maintained at warm temperatures had significantly higher feed conversion ratios (FCR) than urchins maintained at ambient temperatures across all dietary treatment groups (UW: 16.24 ± 2.37; UA: 9.81 ± 0.98) (MW: 4.58 ± 0.44; MA: 2.79 ± 0.23) (FW: 3.02 ± 0.29; FA: 1.91 ± 0.23) (Two sample t-test, $p < 0.05$). *Ulva*-fed urchins exhibited a significantly higher FCR over the course of the study compared to urchins fed the other diets (Tukey, $p < 0.001$) (Figure 18). Urchins fed the formulated feed consistently exhibited the lowest FCR's in the study, significantly lower than the FCR exhibited by *Ulva*-fed urchins (Tukey, $p < 0.01$).

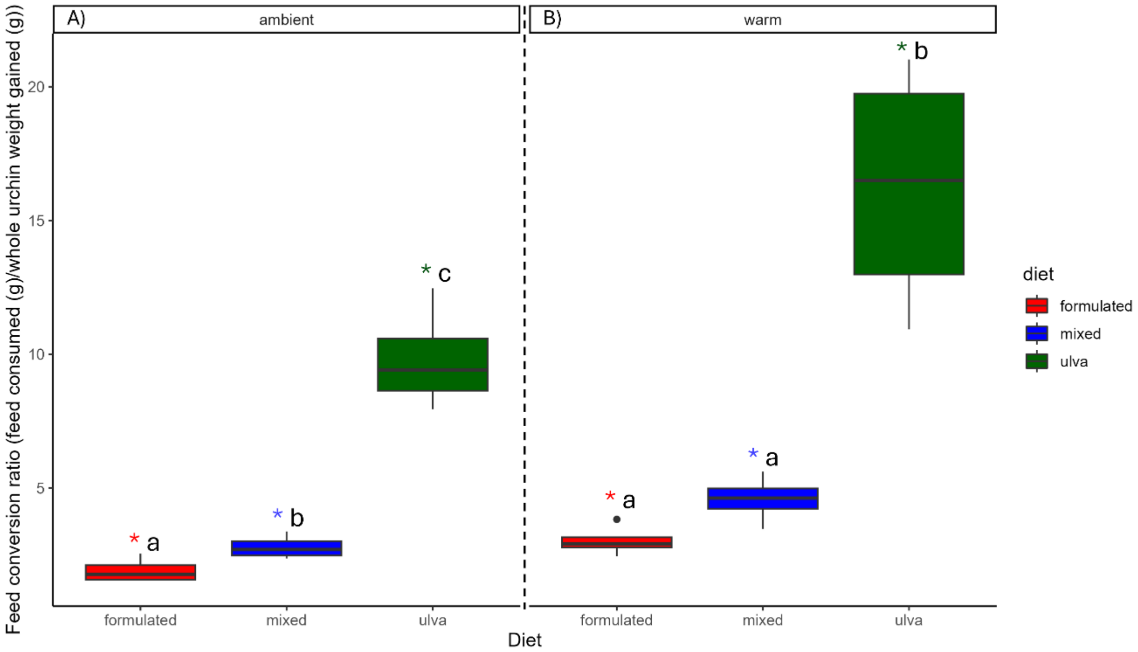

Figure 18. Boxplot of feed conversion ratio (feed consumed (g) / whole urchin weight gain (g) over 23 weeks for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. Different letters indicate statistically significant differences between diets at a specific temperature. Stars indicate statistically significant differences between temperatures for a specific diet.

If moisture content (80%; Cyrus et al. 2014) of fresh *U. lacinulata* is considered, the FCR values for urchins fed *U. lacinulata* and mixed diets are significantly reduced (UA: 9.81 ± 0.98; $UA_{80\%\ reduction}$: 1.96 ± 0.20) (UW: 16.24 ± 2.37; $UW_{80\%\ reduction}$: 3.25 ± 0.47) (MA: 2.79 ± 0.23; $MA_{80\%\ reduction\ of\ U}$: 1.58 ± 0.12) (MW: 4.58 ± 0.44; $MW_{80\%\ reduction\ of\ U}$: 2.02 ± 0.18) (Welch two sample t-test, $p < 0.001$) (Figure 19). This reduction influenced the dietary treatment effects on FCR. Mixed diet fed urchins had the lowest FCR values overall and *Ulva*-fed urchins did not have a significantly higher FCR than all other dietary treatments when moisture content of *U. lacinulata* was considered.

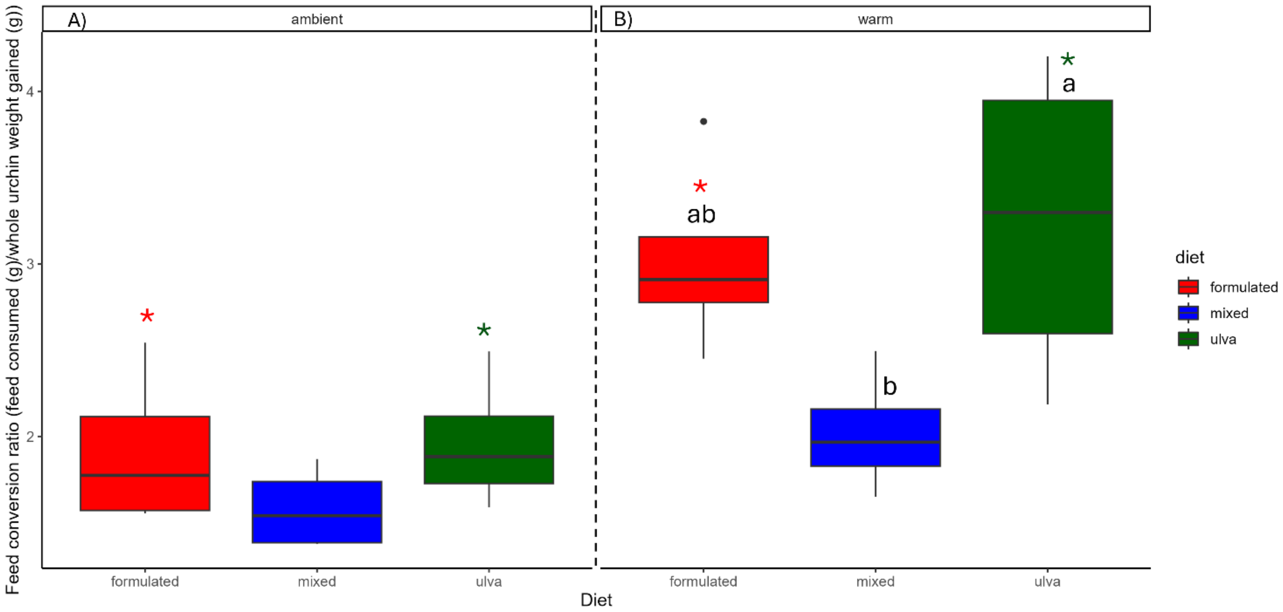

Figure 19. Boxplot of feed conversion ratio (feed consumed (g) / whole urchin weight gain (g) over 23 weeks for the Cape sea urchin, *P. angulosus*, fed different diet treatments (formulated feed with 16% *Ulva*, *U. lacinulata* (with 80% moisture content taken into account)), and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ºC and (B) warm ~19 ºC. Different letters indicate statistically significant differences between diets at a specific temperature. Stars indicate statistically significant differences between temperatures for a specific diet.

3.6. Gonad quantity and quality

3.6.1. Gonad quantity

3.6.1.1. Urchin gonad weight (g)

The *U. lacinulata* (UA $_{\text{week 9}}$: 1.12 ± 0.17 g; UW $_{\text{week 9}}$: 0.91 ± 0.29 g) and kelp (KA $_{\text{week 9}}$: 0.58 ± 0.17 g; KW $_{\text{week 9}}$: 0.67 ± 0.17 g) fed urchins exhibited significantly lower gonad weights (g) at the first measurement timepoint than urchins fed formulated feed (FA $_{\text{week 9}}$: 4.25 ± 0.96 g; FW $_{\text{week 9}}$: 5.43 ± 0.54 g) across both temperatures (Tukey, $p < 0.05$) (Figure 20). It should be noted however, that some kelp fed urchins had insufficient gonad to weigh (Supplementary Figure 2).

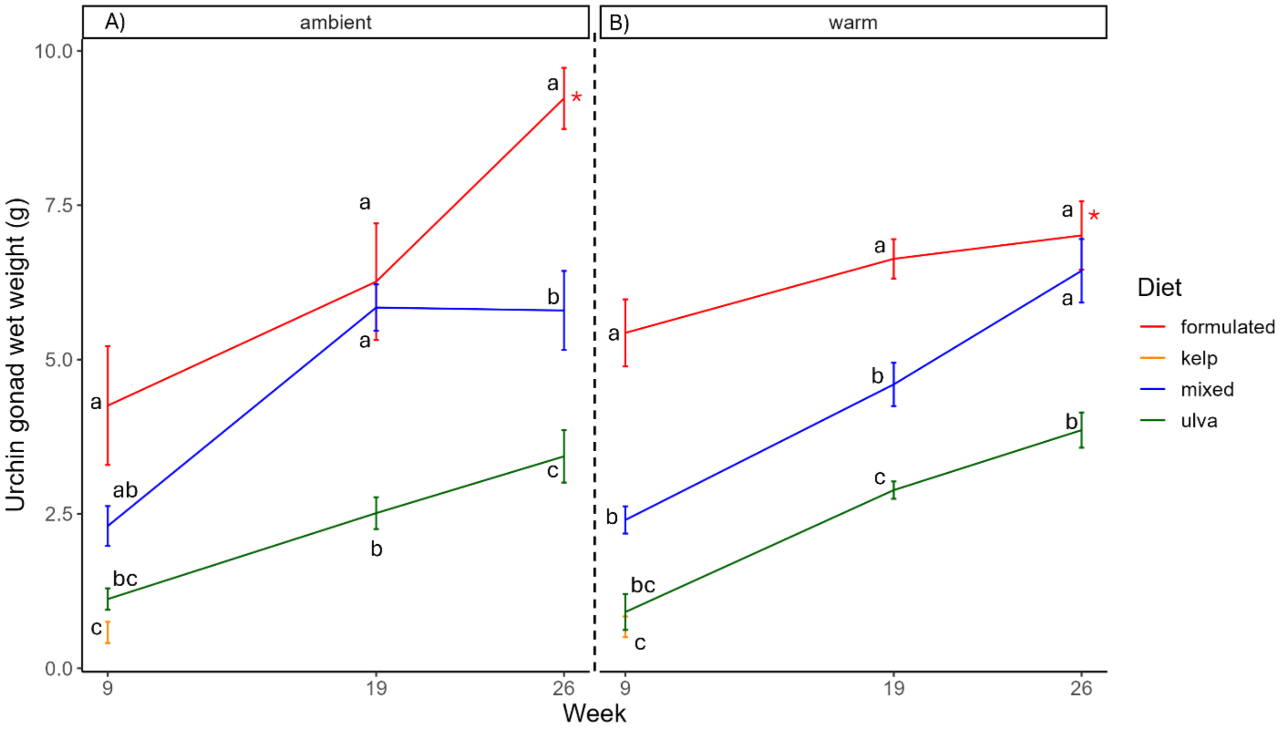

Figure 20. Mean (± SEM) of wet gonad weight (g) for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

The *Ulva*-fed urchins produced significantly lower gonad weights (g) overall (after 26 weeks) (UA $_{\text{week 26}}$: 3.43 ± 0.43 g; UW $_{\text{week 26}}$: 3.86 ± 0.29 g) in comparison with urchins fed other diets (Games-Howell, $p < 0.05$).

Formulated feed-fed urchins produced the highest overall gonad weight (g) in comparison to urchins fed other diets. When maintained at ambient temperatures, formulated-feed fed urchins exhibited significantly higher overall gonad weights than urchins fed other diets (FA$_{\text{week 26}}$: 9.23 ± 0.50 g; MA$_{\text{week 26}}$: 5.80 ± 0.64 g; UA$_{\text{week 26}}$: 3.43 ± 0.43 g) (Games-Howell, $p < 0.001$). When maintained at warm temperatures, formulated feed-fed urchins had significantly lower overall gonad weights (g) than when maintained at ambient temperatures (FW$_{\text{week 26}}$: 7.01 ± 0.55 g; FA$_{\text{week 26}}$: 9.23 ± 0.50 g) (Two sample t-test, $p < 0.01$). Similar overall gonad weights were exhibited by mixed diet and formulated feed-fed urchins at warm temperatures (MW$_{\text{week 26}}$: 6.44 ± 0.51 g; FW$_{\text{week 26}}$: 7.01 ± 0.55 g) (Games-Howell, $p > 0.05$).

3.6.1.2.Gonad somatic index (GSI%)

Temperature had no effect on the GSI (%) of urchins in this study (ANOVA, $p > 0.05$). Kelp and *Ulva*-fed urchin GSI was similarly low in week 9 of the study (UA$_{\text{week 9}}$: 5.61 ± 1.04 %; UW$_{\text{week 9}}$: 3.82 ± 1.19 %; KA$_{\text{week 9}}$: 2.31 ± 0.62 %: KW$_{\text{week 9}}$: 3.00 ± 0.67 %), significantly lower than urchins fed the formulated feed (FA$_{\text{week 9}}$: 18.18 ± 2.93 %: FW$_{\text{week 9}}$: 19.08 ± 1.85 %) (Tukey, $p < 0.01$). By the end of the study (week 26), significantly lower GSI values were observed for *Ulva*-fed urchins than for urchins fed all other diets (UA$_{\text{week 26}}$: 11.57 ± 0.94%; UW$_{\text{week 26}}$: 12.68 ± 0.50 %) (Tukey, $p < 0.001$). Conversely, formulated feed-fed urchins had significantly higher GSI values than urchins fed other diets (FA$_{\text{week 26}}$: 29.06 ± 0.76%; FW$_{\text{week 26}}$: 25.09 ± 1.22 %) (Tukey, $p < 0.05$) (Figure 21).

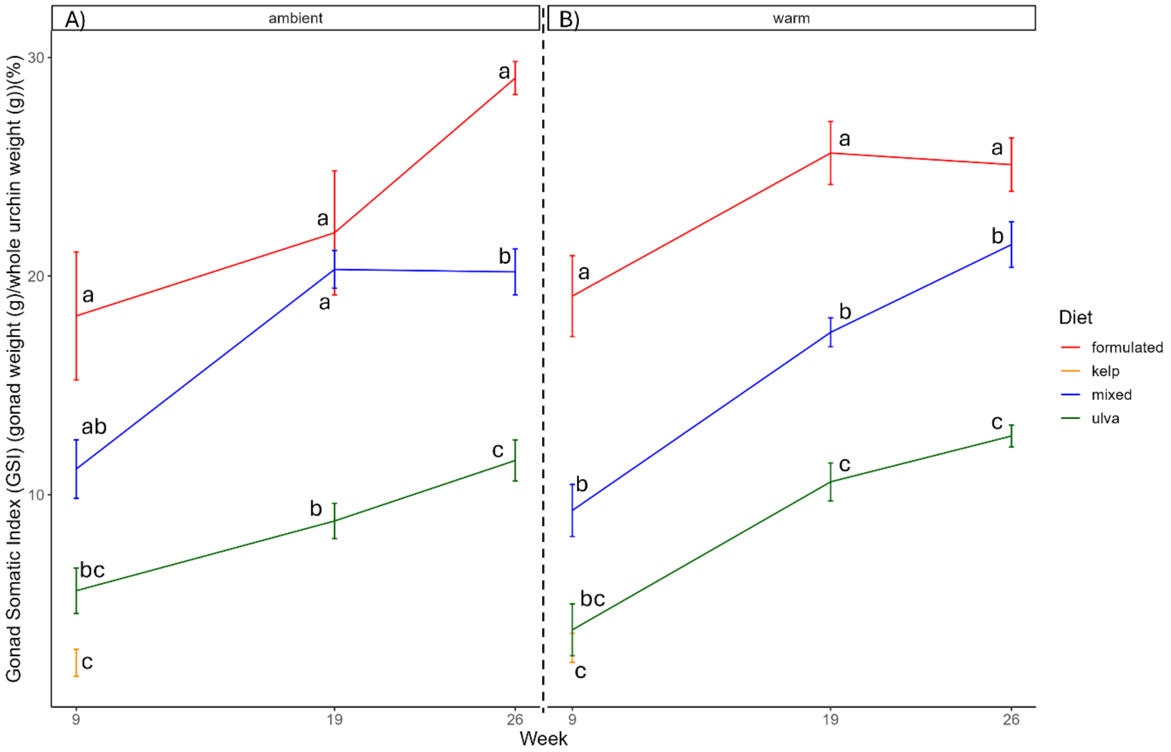

Figure 21. Mean (± SEM) of gonad somatic index (GSI; %), a measure of gonad weight (g) per whole urchin wet weight (g), for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet under different temperature conditions: (A) ambient ~ 16 °C and (B) warm ~19 °C. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

3.6.2. Gonad quality: gonad maturity/development

Temperature had no effect on gonad development for Cape urchins in this study (Fisher exact test, $p > 0.05$). Urchins fed formulated feed, whether exclusively (F) or as part of a mixed feeding regime (M) had significantly less developed gonads than urchins fed natural diets (FA $_{\text{week 9}}$: recovery – premature; UA $_{\text{week 9}}$: premature, mature & spent) (FW $_{\text{week 9}}$: growing & premature; KW $_{\text{week 9}}$: mature) (FW $_{\text{week 19}}$: premature; MW $_{\text{week 19}}$: premature; UW $_{\text{week 19}}$: mature & partly spawned) (MA $_{\text{week 23}}$: premature; UA $_{\text{week 26}}$: partly spawned) (Dunn Bonferroni-corrected, $p < 0.05$) (Figure 22). However, it should be noted that statistical analyses were limited by the low sample sizes in this dataset.

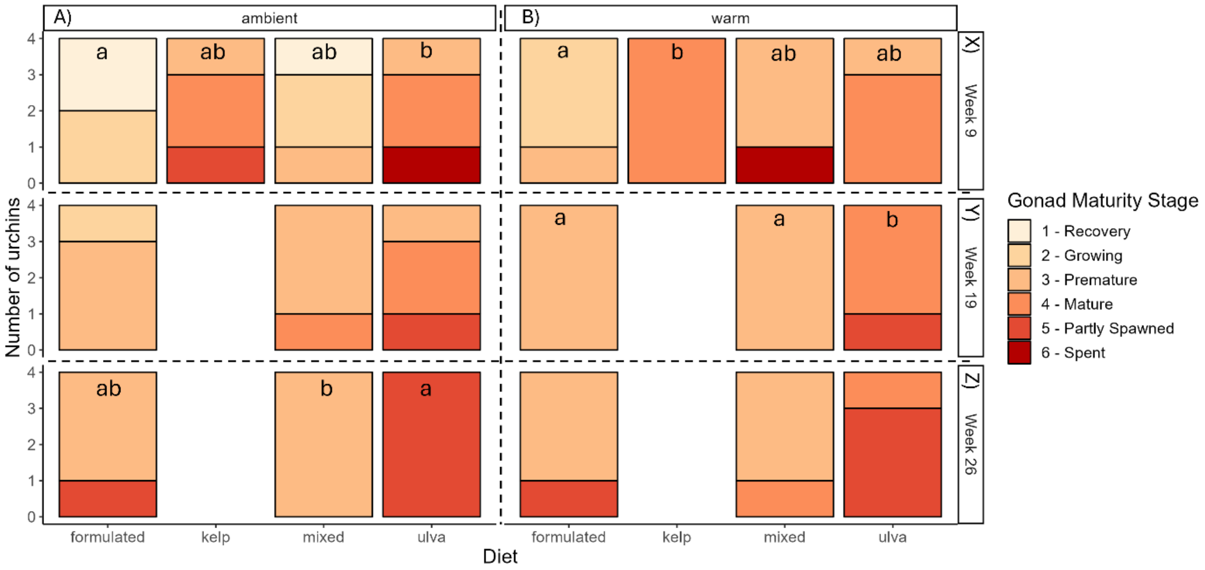

Figure 22. Stacked barplot of gonad maturity stage (1- recovery, 2 - growing, 3 - premature, 4 - mature, 5 - partly spawned, and 6 - spent) for Cape sea urchins, *P. angulosus*, fed different diet treatments (n = 4 per diet per timepoint) (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions: (A) ambient ~ 16 ^{o}C and (B) warm ~19 ^{o}C. Gonad samples were taken after X) 9, Y) 19 and Z) 26 weeks of the experiment. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. No statistically significant differences between temperatures were found for any specific diet and timepoint.

3.6.3. Gonad quality: gonad colour

Temperature had no effect on gonad tissue colouration for the Cape urchin, but dietary effects were evident. By the end of the trial, urchins fed the formulated diet produced significantly paler gonads than urchins fed the other dietary treatments based on visual assessment (Supplementary Figure 3) and gonad colour indices (Figure 23; Figure 24). Paleness corresponded with significantly higher lightness indices (CIE L*) (FA $_{L*}$: 62.22 ± 1.58; UA $_{L*}$: 49.84 ± 3.45) (FW $_{L*}$: 62.65 ± 1.18) (UW $_{L*}$: 49.47 ± 3.27; MW $_{L*}$: 56.08 ± 1.16) (Dunn Bonferroni-corrected $p < 0.05$) and lower redness indices (CIE a*) (FA $_{a*}$: 11.11 ± 0.91; UA $_{a*}$: 25.81 ± 2.76; MA $_{a*}$: 18.61 ± 1.95)(FW $_{a*}$: 12.77 ± 1.31; UW $_{a*}$: 23.69 ± 2.93; MW $_{a*}$: 20.60 ± 1.97) (Tukey, $p < 0.05$).

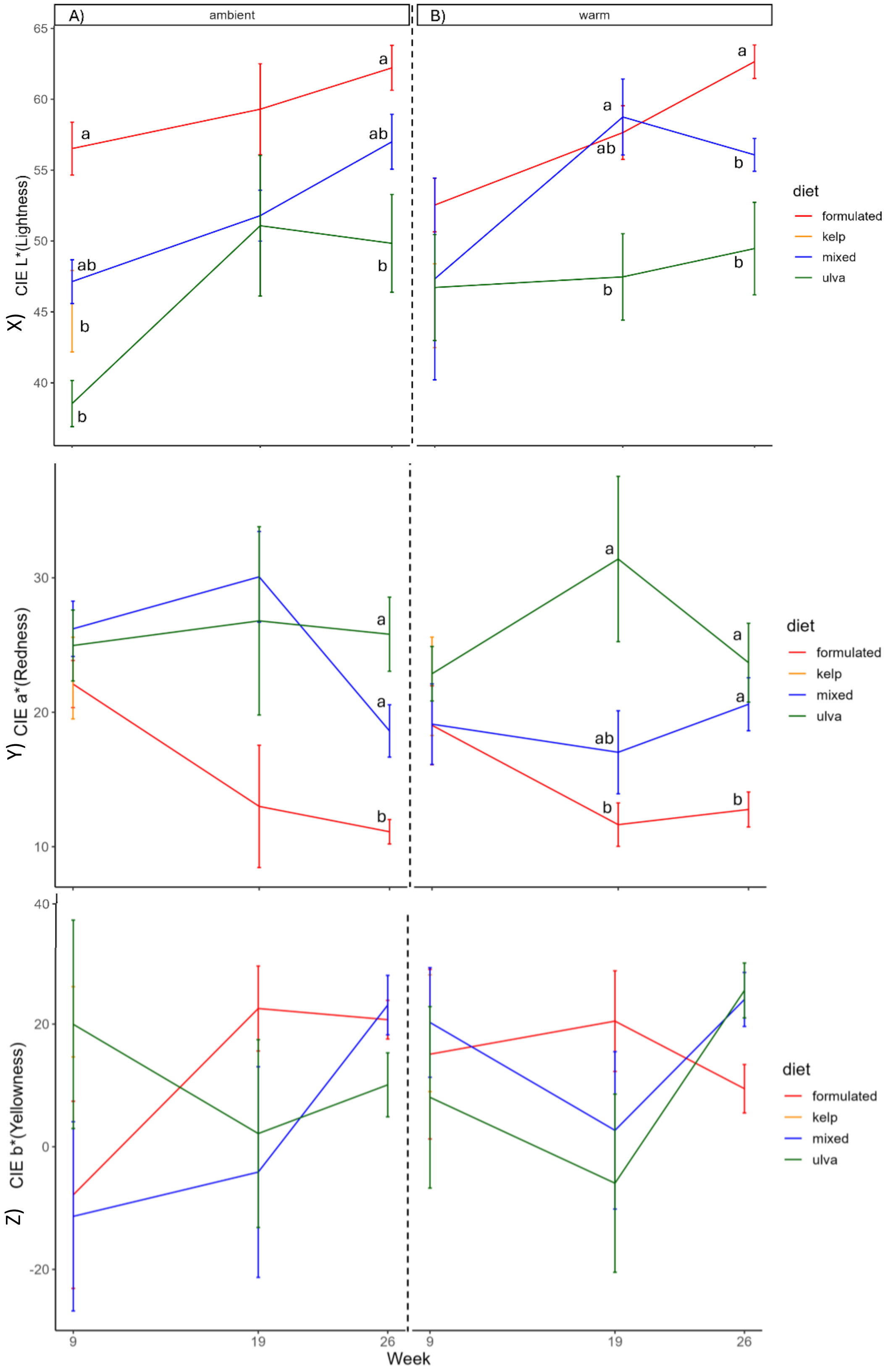

Figure 23. Mean (± SEM) of gonad tissue colouration indices (CIE L*, a* and b*), for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) under different temperature conditions (ambient ~ 16 ºC and warm ~19 ºC). (X) lightness (L*), (Y) redness (a*) and (Z) yellowness (b*). Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. No statistically significant differences between temperatures were found for any specific diet and timepoint.

Yellowness (CIE b*) of urchin gonad tissue did not differ by diet or sex. Gonad tissue colour produced by *Ulva*-fed urchins was more variable than urchins fed other dietary treatments based on the spread of the values exhibited in the principal component analysis (PCA) of gonad tissue colouration indices (Figure 24). Temperature and sex were not considered in the PCA.

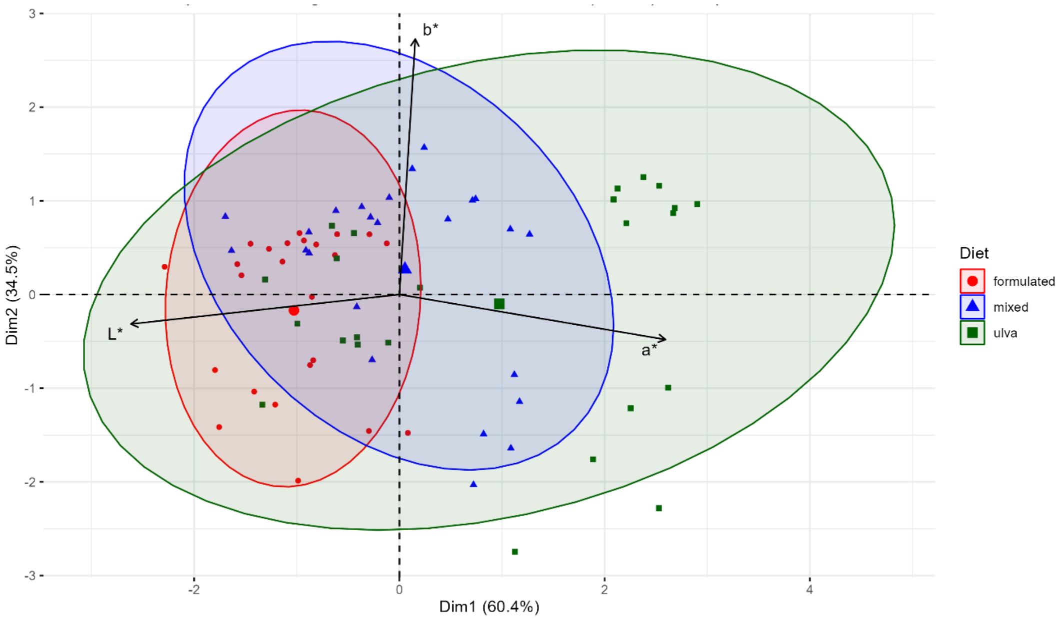

Figure 24. Principal component analysis (PCA) of gonad tissue colouration indices (CIE L*, a* b*) for the Cape sea urchin *P. angulosus* fed different diet treatments (n = 24 per diet) (formulated feed with 16% *Ulva*, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) after 26 weeks, where vectors indicate the relative effect of each colour index on the overall clustering patterns.

Sex had no effect on gonad tissue colour for formulated feed-fed urchins. After 26 weeks, it was difficult to distinguish between male and female based on appearance of formulated feed-fed urchin gonads. The formulated feed-fed urchin gonads were thus not tested for sex effects based on colour in week 26. Sex had a significant effect on gonad tissue colour for urchins fed *U. lacinulata* or mixed diets with male urchins exhibiting significantly paler and less marketable gonads than female urchins based on visual assessment (Supplementary Figure 3) and gonad tissue colour indices (higher lightness and lower redness) (Figure 25). Gonad tissue colour produced by male urchins fed natural diets exclusively (U) or as part of their feeding regime (M) was significantly lighter (male: M_{L*}: 60.93 ± 1.69, U_{L*}: 59.51 ± 2.06; female: M_{L*}: 54.35 ± 1.09, U_{L*}: 43.74 ± 2.46) and less red (male: M_{a*}: 13.23 ± 1.33, U_{a*}: 15.15 ± 1.37; female: M_{a*}: 22.79 ± 1.37; U_{a*}: 30.50 ± 1.83) than gonads produced by female urchins (Two sample t-test, $p < 0.05$).

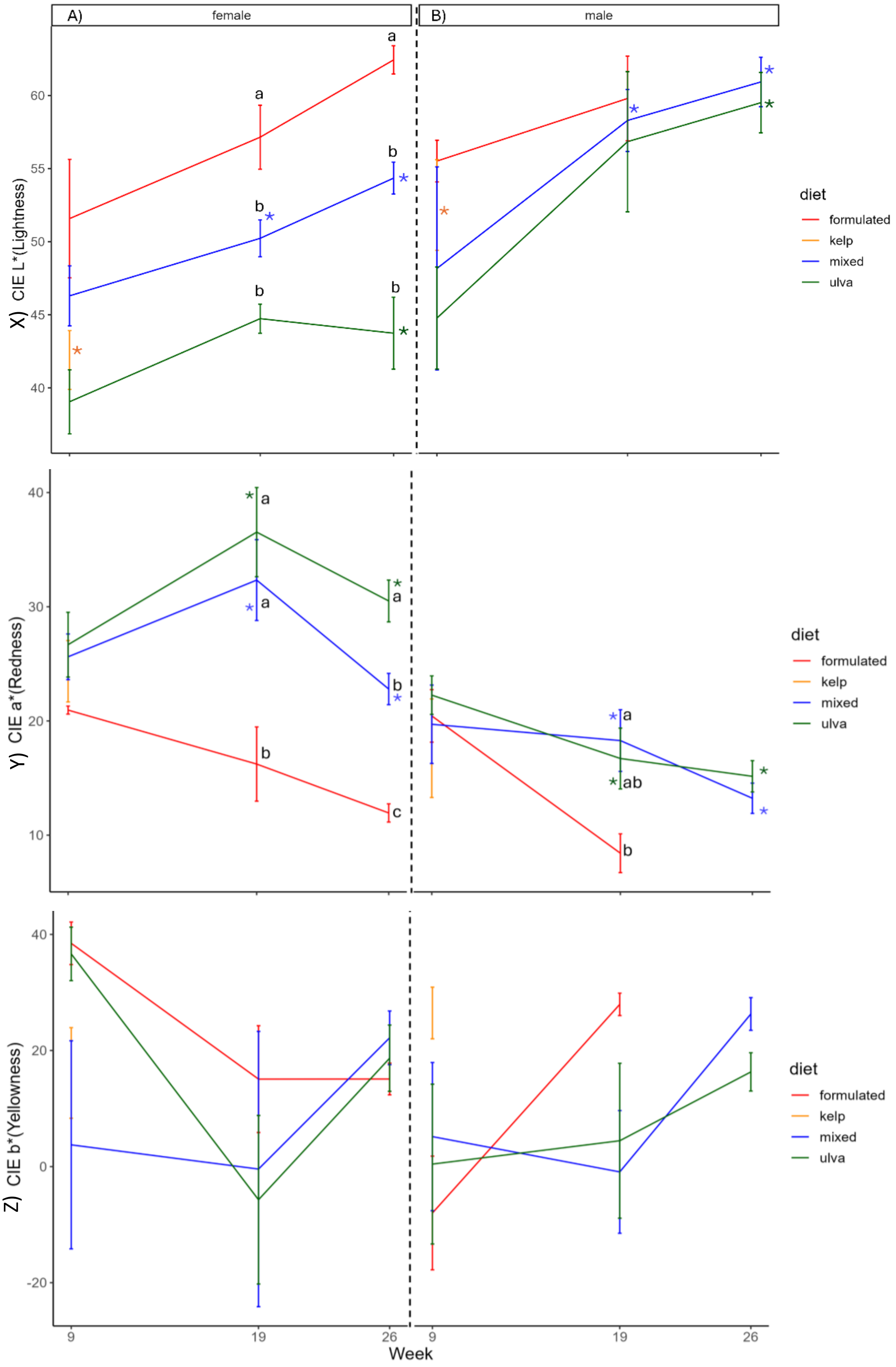

Figure 25. Mean (± SEM) of gonad tissue colouration indices (CIE L*, a* and b*), for the Cape sea urchin *P. angulosus* fed different diet treatments (formulated feed with 16% *Ulva*, *E. maxima* kelp, *U. lacinulata*, and a combination of the forementioned to form a mixed diet) for (A) female and (B) male urchins. Different letters indicate statistically significant differences between diets at a specific timepoint and temperature. Stars indicate statistically significant differences between temperatures for a specific diet and timepoint.

4. Discussion

4.1. *Ecklonia maxima* as a feed for *Parechinus angulosus*

The Cape urchin has been shown to regulate kelp density by grazing on the young sporophytes and kelp debris rather than adult plants (Fricke 1979, Morris and Blamey 2018), however a study by Buxton and Field (1983) reported that *P. angulosus* consumes whole kelp both from floating debris and living plants. The animals selected for this study were collected from a kelp forest region and therefore assumed to regularly consume kelp given the generalist feeding behaviour of sea urchins, including *P. angulosus*.

The results of this study show that *E. maxima* is not a suitable feed for aquaculture of *P. angulosus*. Even though the urchins consumed large amounts of kelp (Figure 17), the animals lost weight (g) (Figure 14) and lacked growth in test diameter (mm) (Figure 12). Gonads were completely absent for some of the urchins, resulting in poor GSI values for the kelp dietary treatment overall (2 – 3 % GSI). For those urchins that remained healthy in this dietary treatment group, gonad quantity was comparable to urchins fed fresh *U. lacinulata* (3 – 6 % GSI). It is however unclear why some urchins in the kelp treatment performed better than others under the same treatment. Overall, high mortality was observed in this group, with less than 70% of the kelp-fed urchins surviving the first 9 weeks of the 26-week study. This survival rate (%) was significantly lower than the survival observed in all other dietary treatments (Figure 10).

An experiment by Anderson and Velimirov (1982) concluded that *E. maxima* is among the least preferred algae species for *P. angulosus* when considering the 13 most common algae in the kelp beds of the west coast of the Cape Peninsula. *Ecklonia maxima* was shown to have significantly higher astringency values and phenol contents, a chemical deterrent which affects the palatability of the algae, than other common algae species (Anderson and Velimirov 1982). A study on the digestive mechanisms of *P. angulosus* showed that the species is unable to digest alginic acid, the main structural polysaccharide of brown algae such as *E. maxima* (Sweijd 1990). Given the high kelp consumption rates, the poor performance of the kelp diet in this study is likely due to the indigestibility of *E. maxima* by *P. angulosus* and not palatability. These findings may provide additional insight into the lack of sea urchin barrens present in South African waters (Figure 3).

The kelp dietary treatment was suspended in week 9 which required a change in the mixed diet feeding regime. From week 10, the mixed diet feeding regime changed to *U. lacinulata* and a formulated feed containing 16% (w/w) dried *U. lacinulata* rotated on a weekly basis. After the removal of kelp from the mixed diet feeding regime, urchins fed the mixed diet gained weight significantly faster than urchins exclusively fed a formulated diet (Figure 15). This finding indicates that the inclusion of kelp in the mixed feeding regime was limiting the somatic and gonad growth of *P. angulosus* in the early stages of the trial. Despite this, mixed diet-fed urchins gained the most weight (g) overall (after 23 weeks) in comparison to urchins fed other diets (Figure 16). The experimental conditions of the mixed diet feeding regime changed during the study and therefore the mixed diet-fed urchins may have performed better should they have not been fed kelp in the first 9 weeks of the 26-week experimental period.

4.2. Dietary effects

In nature, urchins are exposed to a wide variety of resources (omnivorous) to meet their dietary requirements (Lawrence et al. 2013). Natural diets have shown to produce market quality urchin products for other sea urchin species (Pearce et al. 2003, Cyrus 2013). For aquaculture purposes however, limitations exist when feeding natural diets exclusively as harvesting of large quantities has the potential to overexploit natural populations.

Aquacultured *Ulva* has been shown to be effective as an aquafeed for a multitude of species including sea urchins (Cyrus et al. 2015b, Yang et al. 2021, Addis et al. 2023). Additionally, *Ulva* can be grown in IMTA systems, where it extracts and re-uses nutrients in effluent water, thereby increasing the *Ulva* protein content, improving the sustainability profile of aquafarms and reducing reliance on natural seaweed populations (Robertson-Andersson et al. 2008, Bolton et al. 2009, 2016, Nobre et al. 2010, Brand 2023).

Formulated feeds are more consistent in quality, thereby producing consistently larger gonads of uniform quality. Additionally, they have a long shelf-life and can be manipulated to meet the nutritional requirements of the species for which they are intended. In doing so, protein levels in formulated feeds are generally higher than natural feeds which allows for improved gonad yield in echinoculture (Cyrus et al. 2014).

P. angulosus consumed natural diets (*U. lacinulata* and *E. maxima*) at a significantly faster rate, by total weight (g), than the formulated feed (Figure 17). *Ulva* has been identified as an effective feeding stimulant for several commercially important sea urchin species (Cyrus et al. 2015b, Yang et al. 2021, Addis et al. 2023) and results from this study show that *U. lacinulata* can be used as an effective feeding stimulant for *P. angulosus*. Cape urchins fed a mixed diet, consumed formulated feed significantly faster than urchins fed formulated feed exclusively (Figure 17), suggesting that the increased rate of feed consumption when fed *U. lacinulata* every alternate week possibly stimulated their metabolism, resulting in a higher rate of formulated feed consumption (as has been shown with abalone, Brand 2023). The increased feed consumption of macroalgae did not however translate into increased somatic (Figure 11, Figure 14) or gonadal growth (Figure 20). Gonad growth capacity of *Ulva*-fed urchins may have been limited by the low protein levels (~18%; Brand 2023) of *U. lacinulata*. Formulated feed (protein level ~ 26%; Supplementary Table 1) was shown to be a more efficient feed, requiring low quantities to be consumed in exchange for gonad yield resulting in a significantly lower feed conversion ratio (FCR) compared to natural (*U. lacinulata*) and mixed diets (Figure 18). However, taking the moisture content of *U. lacinulata* into account significantly reduced the FCR such that *Ulva*-fed urchins had a similar FCR to formulated feed-fed urchins and mixed diet fed urchins had the lowest FCR values overall (Figure 19).

As shown in other studies, urchins fed a formulated feed exclusively (25 – 29 % GSI) or as part of a feeding regime (mixed diet) (20 – 22 % GSI) produced significantly higher gonad yields than urchins fed *U. lacinulata* (11 – 13 % GSI) (Figure 21) (Cyrus et al. 2014, Onomu et al. 2020, Araújo et al. 2023). In fact, GSI results observed for formulated feed-fed urchins from this study (18 – 19 % GSI $_{\text{after 9 weeks}}$) were higher than reported in previous studies (Pearce et al. 2004: 12.27 % GSI $_{\text{after 6 weeks}}$ & Robinson et al. 2002: 16.61% GSI $_{\text{after 9 weeks}}$) for *Strongylocentrotus droebachiensis*, one of the top commercial sea urchin species (Table 2). However, the quality of these gonads was undesirable as it produced pale coloured gonads (Supplementary Figure 3) with a bitter after taste (personal observation).

Table 2. Mean gonad colouration (CIE L*a*b*) and gonad somatic index (GSI, %) values from this study compared to results from other sea urchin studies.

Species	**Weeks**	**Diet**	**L* (brightness)**	**a* (redness)**	**b* (yellowness)**	**GSI (%)**	**Reference**
Strongylocentrotus droebachiensis	6	(30-39mm size class) Formulated diet: 200 mg beta-carotene/kg dry weight of feed	47.75	26.80	26.85	12.27	(Pearce et al. 2004)
Strongylocentrotus droebachiensis	9	Formulated diet: 250 mg beta-carotene/kg dry weight of feed.	47.5	25.75	17	16.61	(Robinson et al. 2002)
Parechinus angulosus	9	*Ulva lacinulata*	42.63	23.92	14.01	4.72	this study
Parechinus angulosus	9	Formulated feed: 16% *Ulva* inclusion (16F)	54.53	20.57	3.63	18.63	this study
Parechinus angulosus	9	Weekly rotation of *Ulva lacinulata, Ecklonia maxima* and 16F	47.24	22.66	4.46	10.23	this study
Paracentrotus lividus	24	Various (data based on best grade irrespective of diet)	44.4	8.43	20.67	N/A	(Lourenço et al. 2022)
Parechinus angulosus	26	*Ulva lacinulata*	49.66	24.75	17.79	12.12	this study
Parechinus angulosus	26	Formulated feed: 16% *Ulva* inclusion (16F)	62.43	11.94	15.09	27.08	this study
Parechinus angulosus	26	Weekly rotation of *Ulva lacinulata* and 16F	56.54	19.61	23.56	20.81	this study

Urchins fed fresh *U. lacinulata* produced the most desirable gonad tissue colouration from this study based on visual assessment (Supplementary Figure 3). The redness index values for *Ulva*-fed urchins were comparable with those reported for *S. droebachiensis* (Table 2). However, GSI was low and gonad colour profiles of the *Ulva*-fed urchins were highly variable (Figure 24) which may have been influenced by sex (Figure 25). These results are consistent with findings from other studies which have reported that macroalgal diets lack the necessary protein requirements to stimulate high gonad yields, but due to pigmentation in the feed they produce the best gonad colour (Cyrus et al. 2014). *Ulva*-fed urchins also produced more reproductively mature gonads in this study (Figure 22) than formulated feed-fed urchins, likely due to the higher carotenoid levels that could have stimulated gametogenesis (Shpigel et al. 2005, Brink-Hull et al. 2022b).

Protein is understood to be important for enhanced gonad production in sea urchins and considerable effort is placed on improving protein levels in aquafeeds, but understanding optimal protein levels is important as it varies between sea urchin species (Lourenço et al. 2020). When gonad growth is more rapid than carotenoid deposition, pale gonads are produced (Hagen et al. 2008) as observed for formulated feed-fed urchins in this study. Conversely, low protein levels in feeds result in small gonad yields, as observed for *Ulva*-fed urchins in this study. *Ulva* grown in effluent water has been shown to maintain higher levels of protein (up to 25 %) with an amino acid profile comparable to fish meal (Brand 2023). Future studies using effluent-grown *U. lacinulata* as a feed for *P. angulosus* is recommended as this may produce higher gonad yields for the same weight of *U. lacinulata* provided.

Previous studies have found that mixed diet feeding regimes produce the optimal combination of GSI and desired gonad colour (Shpigel et al. 2005). Apart from a better combination of gonad size and colour, urchins fed the mixed diet had the highest survival rates (Figure 10), most consistent somatic growth rate based on whole urchin wet weight (Figure 15) (Table 3) and lowest FCR (when considering moisture contents of *U. lacinulata*) (Figure 19) in comparison to other diets included in this study.

Mixed feeding regimes have also been shown to display more diverse egg fatty acid profiles than exclusively natural or formulated diets when conditioning broodstock of *T. gratilla* (Brink-Hull et al. 2022b). Exposure to a broader array of nutrients, higher ingestion efficiencies and/or improved digestibility because of bacteria introduced through natural feeds are potential reasons for these improvements (Brink 2020). A mixed diet feeding regime is recommended for this species. In addition, increasing the amount of *Ulva* inclusion (up to 20% w/w) (Cyrus et al. 2014) or the addition of concentrated pigmentation in some other way in the formulated feed may produce more favourable gonad tissue colouration, while maintaining the high gonad yields exhibited by urchins fed the formulated and mixed diets in this study.

Feed preference tests under aquaculture conditions would be beneficial for the development of better feeds for this species. While urchins are generalist omnivores in nature, some seaweeds are more preferred over others (Anderson and Velimirov 1982), but what is preferred may not be suitable as an aquaculture feed on a commercial scale. Red algae are among the most preferred by *P. angulosus*, specifically *Pachymenia cornea, Gigartina bracteata, Carradoria virgata, Suhria vittata* and *Gelidium* sp (Anderson and Velimirov 1982). Of these, only *Gelidium sp.* is currently harvested in South Africa (Rothman et al. 2020). *Gelidium sp.* stocks are healthy and current levels of harvesting (80 – 100 tons dry weight) have negligible ecological effects, but harvest areas do not overlap with existing abalone farms where the Cape urchin is more likely to be cultured (Rothman et al. 2020). Other red seaweed species such as *Gracilaria/Gracilariopsis* are grown on abalone farms as feed for juveniles (Rothman et al. 2020). *Gracilaria* has been shown to be a poor feed for *T. gratilla* (Onomu et al. 2020), however it may work as a feed for *P. angulosus*. It is recommended that red seaweed diets are tested for this species, both exclusively and within a mixed feeding regime.

4.3. Temperature effects

The Cape urchin distribution ranges from the cool temperate northern west coast (monthly mean seawater temperatures 11.8 – 13.2°C) to the subtropical east coast (monthly means 21.9 – 24.1°C) (temperature data from Smit et al. 2013). This indicates that Cape urchins have a broad temperature tolerance, an important consideration for sustainable echinoculture. Studies have found high levels of population differentiation resulting in two distinct genetic groupings of *P. angulosus,* one population along the cool temperate west coast (Namaqua province) and the second on the warm temperate subtropical south and east coasts (Agulhas and East coast province) (Muller et al. 2012) (Figure 4). The urchins in this study are assumed to be from the cool temperate west coast population, as they were collected ~ 48 km north-northwest of Cape Point, the population boundary of Muller et al. (2012). The MRA is situated in a major upwelling region, within the Benguela current system on the west coast of South Africa. Upwelling causes temperature fluctuations in the region from September to March, driven by southeasterly winds (Andrews and Hutchings 1980).

The sea urchins maintained under ambient temperature conditions (15.56 ± 0.07 ºC) exhibited significantly lower survival rates (85.53 ± 3.61 %) than urchins cultured in warmer conditions (18.94 ± 0.03 ºC) (97.37 ± 1.21 %) (Figure 10). This may be due to the combined stresses of higher temperature fluctuations (Figure 9) and sedimentation (from visual observations) in the ambient system. When tanks were cleaned, there was noticeably more sediment at the bottom of the ambient seawater tanks. The additional processing and filtering of water supplied to the heated tanks (Figure 7) may have removed more sediment from the incoming water, improving the water quality within this temperature treatment. Water quality is critically important for the health and well-being of aquaculture species. Higher sediment loads may have introduced higher concentrations of nutrients, bacteria or pollutants which may influence oxygen concentrations (data not recorded) within the tank. In future, it is recommended that water quality (oxygen, pH, ammonia, nitrate) is measured to ensure that water filtration levels are controlled for and provide insight on the optimal water quality conditions for *P. angulosus.*

Temperature records for the ambient treatment were significantly more variable in the months of September to December than in the months of June to August. This coincided with the Benguela upwelling season which is driven by southeasterly winds from September to March (Andrews and Hutchings 1980). Upwelling brings cold nutrient-rich water from the depths to the surface, causing fluctuations in surface water temperatures. The urchins used in this study should be accustomed/adapted to the temperature fluctuations in this region, given that they were collected in this upwelling region.

Although the ambient temperature treatment had lower survival rates, urchins in this treatment had faster (test diameter) somatic growth rates on average (Figure 13). The removal of animals, due to mortality, may have skewed the growth data in favour of the ambient temperature treatment, because of the lower stocking densities, given the small sample sizes (n = 19) within each basket at the start of the experiment. It is fair to assume however, that the animals collected for this study may have been better adapted (genetically) to the temperature conditions from which they were collected and therefore they may not be suited to temperatures that are outside of this range. *Parechinus angulosus* is a cold-water species and therefore has a slower growth rate than warm-water species such as the green sea urchin (*Lytechinus variegatus*) but relative to other cold-water species (e.g., *Paracentrotus lividus*) the urchins in this study grew at comparable rates (Table 3).

Table 3. Mean somatic growth rate in terms of specific growth rate (SGR; % growth/day) values from this study compared to results from other sea urchin studies.

Species	**Weeks**	**Diet**	**Temperature**	**Weight SGR (%growth/day)**	**Reference**
Lytechinus variegatus (initial TD 36.0 ± 0.80 mm)	0 – 9	Across diets with various protein contents (PC) (%)	22 ± 2 ºC	9% PC: 0.49 ± 0.07 20% PC: 0.78 ± 0.08	(Hammer et al. 2006)
Parechinus angulosus (initial TD 33.33 ± 0.44 mm)	0 – 8	Across *U. lacinulata (U)*, *E. maxima* (K), formulated (16F) and mixed (M) diets	15.56 ± 0.07 ºC (ambient)	U: 0.16 ± 0.02 K: 0.06 ± 0.04 16F: 0.31 ± 0.01 M: 0.22 ± 0.02	this study
Parechinus angulosus (initial TD 33.33 ± 0.44 mm)	0 – 8	Across *U. lacinulata (U)*, *E. maxima* (K), formulated (16F) and mixed (M) diets	18.94 ± 0.03 ºC (warm)	U: 0.15 ± 0.02 K: 0.04 ± 0.03 16F: 0.22 ± 0.03 M: 0.18 ± 0.03	this study
Paracentrotus lividus (initial TD 33.73 ± 3.90 mm)	0 – 26	Across *U. lacinulata (U)*, formulated (F) diets	~ 18.2 – 22.7 ºC	U: 0.17 ± 0.03 F: 0.29 ± 0.08	(Araújo et al. 2023)
Parechinus angulosus (initial TD 33.33 ± 0.44 mm)	0 – 23	Across *U. lacinulata (U)*, formulated (16F) and mixed (M) diets	15.56 ± 0.07 ºC (ambient)	U: 0.15 ± 0.02 16F: 0.17 ± 0.02 M: 0.23 ± 0.02	this study
Parechinus angulosus (initial TD 33.91 ± 0.40 mm)	0 – 23	Across *U. lacinulata (U)*, formulated (16F) and mixed (M) diets	18.94 ± 0.03 ºC (warm)	U: 0.14 ± 0.02 16F: 0.10 ± 0.01 M: 0.19 ± 0.02	this study

Cape urchins in this study grew significantly larger in test diameter when maintained at ambient temperature seawater than warm temperatures which increased their capacity for gonad growth due to the increased test volume. Urchins cultured in the ambient temperature (FA: 9.23 ± 0.50 g) produced significantly more gonad per urchin (g) than urchins cultured in warm seawater (FW: 7.01 ± 0.55 g) (Figure 20). The age of the urchins used in these trials was however unknown, as they were collected from the wild, and smaller or younger urchins may have had a higher capacity for somatic growth than larger and older urchins. Conversely, older, larger urchins would have a higher capacity for gonad growth and development than younger smaller urchins due them being at more advanced developmental stages. Statistically, formulated feed-fed urchins within the warm temperature treatment group had significantly larger test diameters (mm) than the ambient group at the start of the experiment (Figure 11) but biologically speaking, this difference (~2 mm) is unlikely to have affected the results as urchins above 30mm are considered to be mature (Greenwood 1980) and thus growth capacity is likely to be comparable as the urchins should be within similar developmental stages. Similar patterns were observed for mixed diet-fed urchins which had equal test diameters at the start of the experiment (Figure 11). Urchins fed the mixed diet grew larger tests (MA: 6.73 ± 0.90 mm; MW: 4.50 ± 0.46 mm) (Figure 13) and gained more weight (MA: 6.78 ± 0.61 g; MW: 5.96 ± 0.47 g) (Figure 16) when cultured in ambient seawater than the urchins cultured in the warm seawater.

P. angulosus consumed fresh *U. lacinulata* significantly faster when maintained at warm temperatures than when maintained at ambient temperatures (Figure 17) but this did not result in higher gonad yield (Figure 16). Increased temperature conditions may increase metabolic rate, resulting in higher consumption rate, but gonad growth may have been limited by the low protein content levels (~18%; Brand 2023) of *Ulva lacinulata*. Temperature had no effect on feed consumption rates for formulated feed-fed urchins (Figure 17) but urchins in this dietary treatment gained significantly more weight overall (g) when maintained at ambient temperatures than warm temperatures (Figure 16). The increased temperature may have affected the quality of the formulated feed as formulated feeds tend to disintegrate faster at warm temperatures. The significantly higher leaching rate in the warmer seawater (warm: 25.74 ± 0.98 %; ambient:16.10 ± 0.26 %) indicates feed stability differences which may have affected the quality of the formulated feed consumed. The variable quantity and the quality of the nutrient losses of the formulated feed between the different temperature conditions requires further study.

The relationship between feed consumed and weight gained is defined by the feed conversion ratio (FCR) which is the quotient of feed consumed (g) divided by weight gained (g) by the animal. A low FCR is more economical and efficient than a feed with a high FCR, because this means that for the same amount/weight of feed provided the animal gains more weight. However, FCR is a limited measure of efficiency as it only accounts for the weight of feed inputs and ignores feed nutritional contents (Fry et al. 2018). The Cape urchin gained significantly more weight (g) for the same “ration” of feed when maintained at ambient temperature than when maintained at warm temperatures across all dietary treatments provided in this study. Temperature effects on feed nutritional contents are recommended to develop and refine the optimal aquaculture conditions for *P. angulosus*.

Temperature effects on somatic growth and gonad quality have never been tested for *P. angulosus* prior to this experiment. This study has shown that increased temperature had no effect on gonad development or gonad tissue colouration and negative effects on gonad yield and somatic growth. However, temperature effects on feed quality may have impacted these results and should be investigated further. Based on the results of this study, it is not worthwhile increasing water temperature above ambient for gonad enhancement of *P. angulosus* collected from the cool-temperate west coast. Increasing the temperature of culturing conditions did not improve the market quality or increase the yield of *P. angulosus* gonad products and would incur additional heating costs for an aquafarm situated in a cool seawater temperature region. Urchins collected from the warm temperate – subtropical population of *P. angulosus* (warm south and east coasts of South Africa) (Muller et al. 2012), may have a higher temperature tolerance than the cool temperate west coast population and thus may perform differently under the experimental conditions of this study. Further studies comparing somatic and gonad growth for animals collected from different *P. angulosus* populations identified along the South African coast, which are possibly genetically adapted to the different temperature regimes, under aquaculture conditions is required.

4.4. Age effects

Sexual maturity influences temperature effects on metabolic (respiration) rate of the Cape urchin and consumption and growth rates thus decrease with age as urchins reach sexual maturity (Greenwood 1980). Age and size are important factors governing sexual maturity. Marine invertebrates become mature when they reach a certain size (Chalienko et al. 2021). Urchins for this study were deemed to be of a sexually mature size (above 30 mm in diameter) (Greenwood 1980). A full-life cycle grow-out which compares both the cool – temperate and warm temperate – subtropical populations of this species at different stages of the life cycle is recommended. This would standardise age and allow for the assessment of sexually immature (smaller than 17 mm) (Greenwood 1980) organisms and allow for more control over size classes and weights of urchins. Moreover, potential genetic differences in growth traits between animals can be controlled for if they have the same progenitors/parent pairs. A full-life cycle grow-out may improve our understanding of diet and temperature effects on optimal somatic and gonad growth and development of the Cape urchin.

4.5. Sex effects

In contrast to *T. gratilla* (Cyrus 2013) the quality of *P. angulosus* gonads (tissue colour) was highly influenced by sex for urchins fed natural diets. Gonad tissue colour of male urchins fed a diet with high beta-carotene levels (*U. lacinulata*) was less marketable than females based on visual assessment (Supplementary Figure 3). Echinenone is the xanthophyll pigment primarily responsible for urchin gonad tissue colouration which is derived from carotenes (beta-carotene) which are present in seaweeds. This explains why sex-specific differences were not found in urchins fed formulated feeds as carotenoid levels are lower. These sex-specific differences in gonad tissue colouration are due to echinenone being incorporated into eggs and not sperm (Hagen et al. 2008). Since males do not require echinenone for sperm production, they are unable to convert excess beta-carotene present in their diets into echinenone during gametogenesis. This causes carotenoids to build up, leading to dark (undesirable) gonad tissue colouration (Hagen et al. 2008).

Gonad developmental stage has been shown to affect carotenoid content in gonad tissue (Borisovets et al. 2002) but the gonads produced at the end of this study were in similar developmental stages. There is no knowledge of sex-related differences on feed consumption rate, but sex-specific differences in carotenoid metabolism (i.e., uptake, utilisation, deposition) to form echinenone have been demonstrated for certain sea urchin species *(S. droebachiensis*; Hagen et al. 2008) (*Paracentros lividus*; Lourenço et al. 2022) (*Psammechinus miliaris;* Suckling et al. 2011) — supporting the observations of this study, where female produced gonads of higher market quality than male gonads. Female gonads have a higher capacity for gonad tissue colouration, due to the high number of nutritive phagocytes present in comparison to males, which increases their potential for carotenoid storage (Lourenço et al. 2022).

In addition to tissue colour differences, previous studies have found taste and texture differences between sexes with females being preferred (Baião et al. 2021). Lipid and fatty acid profiles differ between sexes as successful oocyte development requires higher quantities of lipid and fatty acid reserves than the development of highly mobile sperm (Díaz de Vivar et al. 2019). Resources would therefore be better spent exclusively culturing female urchins when fed natural or artificially pigmented diets (Hagen et al. 2008). However, it is not possible to identify the sex of the urchin prior to dissection or spawning. If it is possible to manipulate culturing conditions (genetically or environmentally) to produce more female urchins, this could have significant economic benefits for the industry. Further development on identification, understanding and manipulation of sea urchin sex ratios is needed. Alternatively, the development of products which can utilize the less aesthetically pleasing male gonads (e.g., in pastes, sauces etc.) is a possibility (Stefánsson et al. 2017).

4.6. Future considerations

4.6.1. Taste and texture effects

As with any consumable product, taste and texture of sea urchin gonads are key purchase determinants (Baião et al. 2021). The lack of taste and texture assessments restricts this study from making conclusive remarks on the potential of this species as a new entrant for the sea urchin market. These are two critical factors which affect the market value of sea urchin gonads. Quantitative analyses of taste involve an analysis of the composition of umami, sweet and bitter-tasting amino acids in the gonad tissues (Angwin et al. 2022). Quantitative analyses of texture involve compression tests, in accordance with AACC standard firmness methods (AACC 2009). Taste and texture of the Cape urchin gonad products should be investigated in future using these or similar quantitative methods to allow for comparison with other commercially relevant sea urchin species. An analysis of the nutritional profile as well as sensory profiling by experienced ‘uni’ market professionals (e.g., chefs, harvesters, traders, researchers, consumers) would also provide valuable insight and benefit the marketability of *P. angulosus* products (Baião et al. 2022).

4.6.2. Sea urchin waste

Should the Cape sea urchin gonads be desirable for ‘uni’ markets it would be feasible to explore alternative uses of the inedible portions of the organism to improve the sustainability of the industry. Gonads make up approximately 10 – 30 % of the whole urchin weight on average (Table 2). Due to the high proportion of inedible parts of the sea urchin, regarded as waste, there have been studies investigating uses for exoskeletons and spines of other sea urchin species (Marzorati et al. 2021, Zilia et al. 2021, Melotti et al. 2023). These studies suggest that biocompatible marine collagens, calcium supplements (for agricultural feeds), magnesium and antioxidants can be extracted from the inedible parts of sea urchins. In addition to the use of *P. angulosus* faecal matter within an IMTA system to enhance juvenile growth (Brink-Hull et al. 2022a, Cloete 2022), the extraction of these valuable compounds could provide additional revenue streams through pharmaceutical, agricultural and cosmetic industries. A circular (produce, consume, renew, recycle), rather than a linear (produce, consume, dispose), business model for urchins has potential (Zilia et al. 2021). Future research on product development using the inedible portions of *P. angulosus* would promote sustainable development of the industry.

4.6.3. Sea urchin transportation and processing

Limited local markets for sea urchin gonad products exist and market research would be required for *P. angulosus* as a new seafood product in South Africa. The production of marketable gonads is wasted if the products are not able to reach the market. Cape urchin gonad products would need to travel long distances if they are desired by Asian markets and stress during live transport may influence gonad quality. Processing and/or preservation methods of gonad products may influence taste and texture, and this would require further study. Optimal transportation of sea urchins to markets adds a layer of complexity, and in some cases an additional impediment, to the growth of sustainable echinoculture (Bennett et al. 2024).

There is a well-established trade route between South African abalone (*Haliotis midae*) farms and Asian markets. Live abalone transportation by air freight has been studied and optimised (O'Omolo et al. 2003, Vosloo and Vosloo 2006) and live urchins could be transported using similar methods via the same trade routes. Live urchins are in highest demand (

Figure 2) and are up to three times more valuable than fresh or frozen 'uni' (Bennett et al. 2024). Live abalone is transported on ice in plastic bags, containing 100% O_2 humidified with seawater, within polystyrene containers. Live *S. droebachiensis* are air freighted from Norway to Japan dry (out of water) in polystyrene foam boxes with frozen gel packs to maintain a low temperature (Bennett et al. 2024). These dry transport methods are suitable for short (i.e., hours or days) logistic chains which have a low risk of mortality due to desiccation (Bennett et al. 2024). A study on *T. gratilla* from the tropical Indo-Pacific and *Heliocidaris* *erythrogramma* from temperate Australia showed that these species, which have an ambient temperature of ~ 27 ºC, can be transported using dry transport methods for up to 24 hours when temperature was kept relatively cool at ~12 ºC (Bennett et al. 2024). *Parechinus angulosus* has an ambient temperature similar to abalone of ~15ºC and therefore keeping the urchins relatively cool during transport may require similar methods used by the abalone industry. Considering the long distance (~ 36 hours flight time) between South Africa and Asian markets (Vosloo and Vosloo 2006), the effects of transportation on the quality of *P. angulosus* gonad urchin products requires investigation.

Optimal transport and or preservation processes (e.g., in brine, alcohol or salt) which can maintain the quality of *P. angulosus* gonad products long enough to reach Asian markets is a critically important consideration when assessing the feasibility of the Cape urchin as a new South African aquaculture species. Transport and processing effects on gonad quality (taste, texture and colour) should be investigated.

5. Conclusion and recommendations

Parechinus angulosus has the potential to produce market quality gonad products and be a new aquaculture species for South Africa. The broad temperature tolerance range and wide distribution make *Parechinus angulosus* an accessible and resilient species for South African sea urchin aquaculture investors and existing aquafarms (e.g., abalone, mussels) in areas along the cooler regions of the coast. The species can be cultured successfully within an IMTA system with juvenile abalone (land-based). An IMTA system with mussels (sea-based) on the west coast of South Africa may also have potential and requires further research (Cook and Kelly 2009).

The development of a good diet is critically important for the commercial potential the species to be realised under aquaculture conditions. Natural feeds enhanced gonad maturity, colour and taste; formulated feeds enhanced gonad yield. A balance of quality and quantity was found with a mixed diet feeding regime. The improvement of a formulated feed, through the addition of concentrated pigmentation or more macroalgae, is required in addition to the identification of more suitable natural feeds. Future research to develop an optimal feeding regime by using formulated feeds for growth and natural diets to improve colour, or formulated feeds that incorporate higher proportions of algae such as *Ulva* would produce marketable gonad colour without compromising on gonad yield, improving profitability.

The Cape urchin has the potential to produce marketable quality gonad of commercial interest through gonad enhancement of wild caught sea urchins. However, given the ecological importance of *P. angulosus* in local kelp forest ecosystems, as opposed to urchin barren causing species in other regions (Figure 3), if an industry were to develop, production would ideally be through a full life cycle grow-out rather than harvesting natural populations for gonad enhancement. The added benefits of a full life cycle grow-out is the ability to account for age and genetic variability effects on somatic- and gonad growth, as well as gonad development and quality, a full life cycle grow-out study is recommended for future research. Sex was found to have a significant effect on gonad quality, with female gonads being more marketable. Further development on the identification, understanding and possible manipulation of sea urchin sex ratios is needed, and/or there should be a focus on the development of other uses for male gonads. Lastly, sensory (taste and texture) assessments are required to further assess the commercial potential of this species as these are two critical factors which affect the market value of sea urchin gonads. This study has shown that the Cape urchin gonad product is highly influenced by diet and sex and more research is required for *P. angulosus* to reach its full potential as a new aquaculture species for South Africa.

6. References

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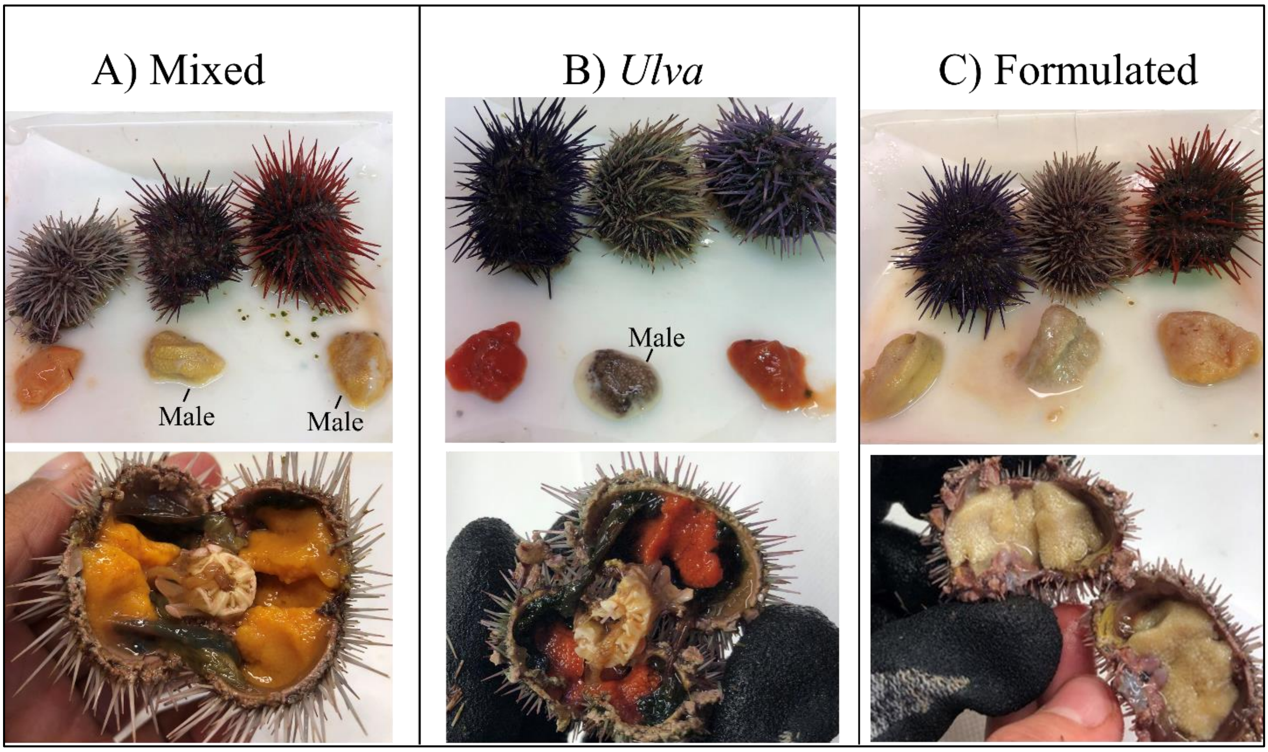
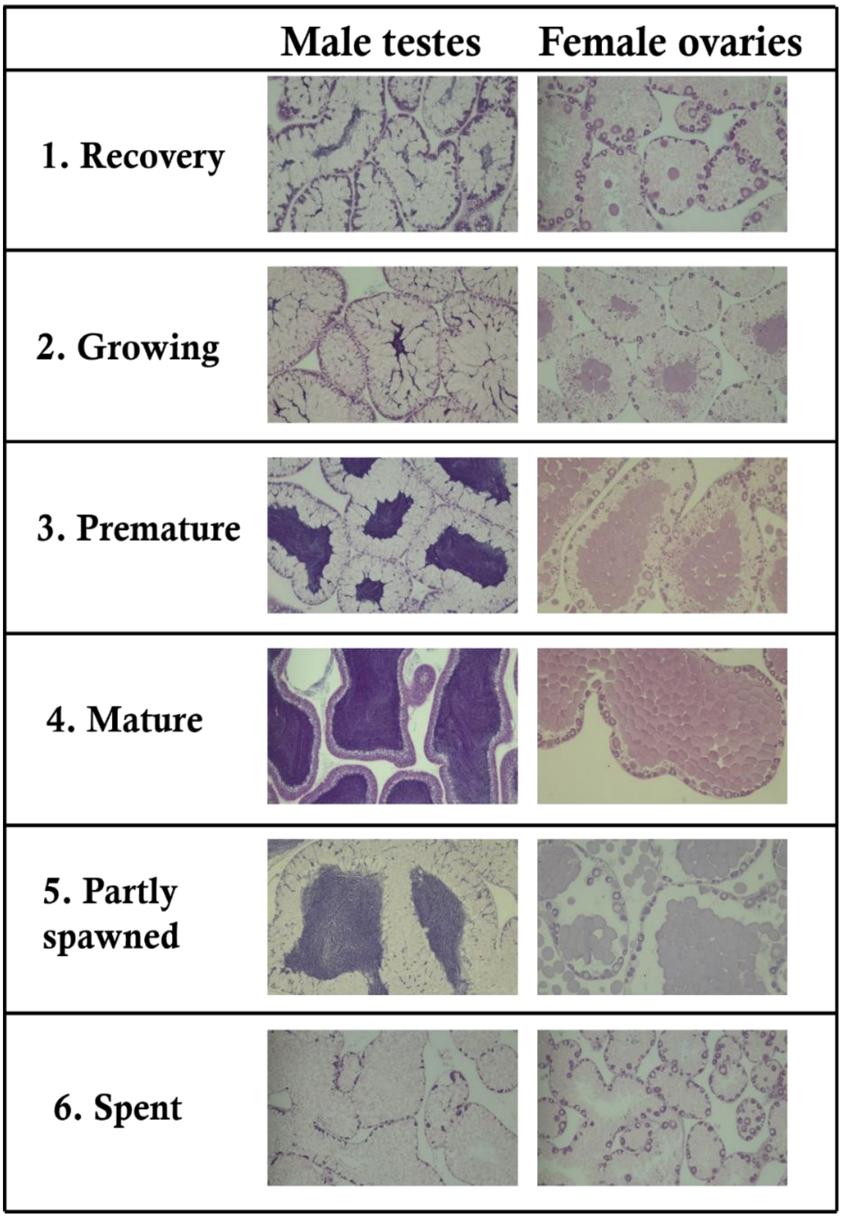
7. Appendix: Supplementary figures and tables

Supplementary Figure 1. Photograph of kelp-fed sea urchins, *P. angulosus*, with spine loss, which were removed from the experiment and euthanised (Aimee Cloete).

Supplementary Figure 2. Photograph of a kelp-fed sea urchin, *P. angulosus*, which had insufficient gonad to quantify after 9 weeks (Aimee Cloete).



Supplementary Figure 3. Photographs of gonads produced by A) mixed, B) *Ulva lacinulata* and C) formulated feed treatment regimes.



Supplementary Figure 4. Gonad histology of *P. angulosus* male testes and female ovaries depicting the respective gonad maturity stages: (1) recovery, (2) growing, (3) premature, (4) mature, (5) partly spawned, and (6) spent.

Supplementary Table 1. Approximate nutrient analysis summary for feeds used (*Ecklonia maxima*, *Ulva lacinulata*, formulated feed with 16% w/w *Ulva* inclusion) based on data provided from other studies (n.d = not determined).

Nutrients (% dry weight)	***Ecklonia maxima*** [a]	***Ulva lacinulata*** [a]	**Formulated feed (15U)** [b]
Protein	11.00	18.31	26.38
Fat	1.16	0.38	3.52
Moisture	79.39	15.30	9.45
Ash	19.41	32.66	11.44
Gross energy (MJ/kg)	n.d	9.44	16.22
Fibre	41.34	6.02	5.44
Carbon/Carbohydrate	33.82	27.33	43.76

[a] as described by Smith 2007

[b] as described by Cyrus et al. 2014, the 16U formulated feed used in this study is assumed to have similar nutrient contents to the 15U formulated feed.

Supplementary Table 2. Formulated feed composition (in g per kg dry matter) prior to the inclusion of *Ulva* as described by Cyrus et al. 2014

Ingredients	**Ratio (g/kg)**
Maize (extruded)	321
Wheat bran	321
Fish meal (65)	153
Soybean (44)	153
Di-calcium phosphate	18.40
Lecithin (de-oiled)	13.80
Vitamin and mineral premix	11.00
Oil (fish)	9.63
Total	1000