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Long term effects of altered pH and temperature on the feeding energetics of the Antarctic sea urchin, *Sterechinus neumayeri*.

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Running Title: Long term OA energy budget

Summary

This study investigated the effects of long-term incubation to near-future combined warming (+2 °C) and ocean acidification (-0.3 and -0.5 pH units) stressors, relative to current conditions (-0.3 °C and pH 8.0), on the energetics of food processing in the Antarctic sea urchin, *Sterechinus neumayeri*. After an extended incubation of 40 months, energy absorbed, energy lost through respiration and lost as waste were monitored through two feeding cycles. Growth parameters (mass of somatic and gonad tissues and the CHN content of gonad) were also measured. There were no significant effects of combined ocean acidification (OA) and temperature stressors on the growth of somatic or reproductive tissue. Despite more food being consumed in the low temperature control, once food processing and maintenance costs were subtracted, there were no significant effects of treatment on the scope for growth. The biggest significant differences were between food consumed during the two feeding cycles. More food was consumed by the low temperature (0°C) control animals, indicating a potential effect of the changed conditions on digestive efficiency. Also in November, more food was consumed, with a higher absorption efficiency which resulted in a higher scope for growth in November than September, which may reflect increased energetic needs associated with a switch to summer physiology. The effect of endogenous seasonal cycles and environmental variability on organism capacity is discussed.

Key-words carbonate saturation; climate change; echinoderm; energetics; ocean acidification; physiology; resilience

Introduction

To predict future patterns of biodiversity it is essential to understand the mechanisms that will determine organism vulnerability. Of the physical factors affecting ectotherms, temperature is one of the most extensively studied and global patterns of thermal tolerance have improved our understanding of how environment correlates with physiological capacities (Gaston *et al.* 2009; Sunday, Bates & Dulvy 2011; Peck *et al.* 2014). Warming oceans increase the body temperature of marine ectotherms, which alters the rates of all organism biochemical reactions (Hochachka & Somero 2002). The vulnerability of organisms to warming therefore depends on the characteristics of their thermal tolerance windows and both their physiological plasticity and adaptive capacity to alter these windows (Angilletta 2009; Somero 2012). Whilst the distributions of many marine species are shifting in response to the rate of environmental warming (Appelhans *et al.* 2014), the effects of temperature do not work in isolation. Within the marine environment the interacting effects of increasing temperature and ocean acidification are predicted to be two of the key factors driving range shifts (Pörtner 2012).

Ocean acidification is likely to have wide ranging effects on marine invertebrates, particularly those with calcified skeletons. The absorption of anthropogenic carbon dioxide into shallow seas is leading to a reduction in carbonate mineral saturation states, particularly aragonite (McNeil & Matear 2008; Fabry *et al.* 2009). This could either result in altered skeletal structure (Bray, Pancucci-Papadopulou & Hall-Spencer 2014), potentially altering predator prey interactions (Watson *et al.* 2012), or, if skeletal structure is maintained, the costs of producing skeleton may increase (Wood, Spicer & Widdicombe 2008). Ocean acidification may also alter the balance of metabolic costs, as extra energy is required to

maintain the homeostasis of inner body fluids against hypercapnia (internal CO_2) and acidosis (reduction of internal pH; Wood 1993; Pörtner, Bock & Reipschlager 2000; Melzner *et al.* 2009; Spicer *et al.* 2011). However, more subtle changes have been identified, which would not necessarily be predicted by the effects of calcium ion concentration on skeletal structure, such as the ability to detect prey, aerobic scope and behaviour (Munday, Crawley & Nilsson 2009; Munday *et al.* 2009; Dixson, Munday & Jones 2010).

The shallow seas around the Antarctic Peninsula have one of the least variable thermal regimes on the planet, with a 3-4 °C annual sea surface temperature range (Peck, Convey & Barnes 2006). Consequently, many Antarctic marine species are stenothermal, with generally poor capacities to cope with elevated temperatures (Pörtner, Peck & Somero 2007). Acclimation is known to take longer in Antarctic marine invertebrates (Morley *et al.* 2011; Peck *et al.* 2014) and their slow generation times and lower fecundity are expected to reduce the capacity for adaptive change (Somero 2010; Peck 2011; Peck *et al.* 2014). Carbon dioxide is more soluble in cold waters (Guinotte & Fabry 2008) and so high latitude oceans are also expected to be amongst the first to become under-saturated with respect to calcite and aragonite (McNeil & Matear 2008). The effects of temperature and ocean acidification are therefore expected to have greater effects on Polar shallow water communities than at lower latitudes (Hofmann *et al.* 2010). Recent studies have, however, shown that aragonite saturation state varies markedly, between 0.8 and 3.9 off the Western Antarctic Peninsula (WAP; Bjork *et al.* 2014; Collard *et al.* 2015). This high natural variability may result in species from the WAP having the physiological capacity to cope with variation in carbonate saturation state.

The Antarctic sea urchin, *Sterechinus neumayeri*, is an important component of shallow water ecosystems throughout the Southern Ocean (Fabry *et al.* 2009). *S. neumayeri* are omnivorous, benthic pioneer species, occurring in high densities in recent iceberg scours, where a large portion of their diet comes from scavenging on dead organisms. Any major effect of future conditions on this keystone species could lead to dramatic shifts in Antarctic benthic food webs. Due to the high Magnesium calcite composition of echinoid skeletons they are a taxonomic group which was predicted to be particularly susceptible to the effects of ocean acidification (Sewell & Hofmann 2011), although recent studies have shown that some echinoids are quite resilient (Wittmann & Pörtner 2013; Collard *et al.* 2015; Suckling *et al.* 2015). Studies are therefore required to determine the capacity of *S. neumayeri* to future temperature and ocean acidification allowing predictions to estimate their future role as a key-stone species in shallow Southern Ocean. In our previous investigations of the same *S. neumayeri* used in this current study, whilst reproduction and larval development were partially acclimated, adult somatic, skeletal growth and reproduction were fully acclimated to altered conditions after 8 months (Suckling *et al.* 2015). In our previous study, *S. neumayeri* were fed *ad libitum* and food consumption was not recorded (Suckling *et al.* 2015). The aim of the current project was therefore to determine if there were subtle changes in the energetics of the same adult Antarctic urchins, *Sterechinus neumayeri*, after a further 16 months incubation (40 months in total) to a combination of elevated temperature and pCO_2 treatments, which would not have been detected using techniques in our previous study (Suckling *et al.* 2015). Specifically, food consumption and the energetic costs of maintenance and food processing were investigated to examine if acclimation to predicted future conditions resulted in any changes in the energy budget, which could influence the scope for growth and long-term resilience to altered environmental conditions.

Material and methods

Animal collection and incubation

Adult *Sterechinus neumayeri* were collected by SCUBA divers in the austral summer of 2008-2009 from 5-10 m depth at South Cove, Ryder Bay, Antarctic Peninsula (67°34' S, 68°08' W). Environmental conditions in Ryder Bay at 5-10m depth consist of seawater temperatures that range from -1.8 to +2.0 °C, however, temperatures rarely exceed +0.5 °C and salinity remains between 32.5-34.5 (Venables, Clarke & Meredith 2013). The animals were transported to the UK and held in the British Antarctic Survey 0 °C re-circulating aquarium in Cambridge for approximately 2 months before being introduced to the re-circulating CO_2 microcosm system (adapted from Widdicombe and Needham (2007) and fully detailed in Suckling *et al.* (2015)). Seawater was transported to Cambridge from the North Sea which had an aragonite saturation state (0.75) slightly lower than the 0.8 to >3 range, but a pH of 8.0 which is within the range of typical values (7.6 to 8.3), for the Western Antarctic Peninsula (Collard *et al.* 2015; Hauri *et al.* 2015). The treatments used in this study were based on the IPCC 'business-as-usual' scenario with the forecasted reduction of 0.3 to 0.5 pH units in oceanic surface waters by the year 2100 (Barbarino & Lourenco 2009) and a predicted rise in surface sea temperature of 2.0 °C. The four treatment combinations were: 1) Low temperature control, present day temperature (-0.3 °C) and pH (pH 8.0); 2) High temperature control, elevated temperature (2 °C) and current pH (pH 8.0); 3) -0.3 pH, elevated temperatures (2 °C) and moderate acidification (pH 7.8) and 4) -0.5 pH, elevated temperature (2 °C) and high acidification (pH 7.5). Urchins were incubated in microcosms under the 4 treatment conditions for 40 months (beginning June 2009).

In the two microcosms with reduced pH treatments (200 L), UV disinfection and 50 μm filtered seawater was delivered to 80L closed cylindrical mixing tanks. CO_2 gas (British Oxygen Company) was introduced via a ceramic diffuser using an Aquamedic pH controlled computer and electrode system and mixed with seawater by an Aquamedic ocean runner power head 2000. Treated seawater was gravity fed to each experimental tank at a rate of 0.56 ± 0.03 L min^{-1}. The pH control mesocosm had a similar header tank, without a pH computer controller, but with an Aquamedic ocean runner power head 2000. The low temperature control animals were kept in a recirculating aquarium facility with identical pre-treatment of water.

Seawater pH was initially at control levels in all tanks, with the urchins acclimated to these tank conditions for 14 days prior to starting the incubations. The pH of the sea water in selected tanks was then gradually decreased in equal twice daily increments over a period of 3 days until the desired pH target was achieved.

Water chemistry

Temperature was recorded daily for all treatments (ºC; Digital Testo 106) and the room temperature adjusted as required. Once weekly temperature, salinity (Tropical Marine Centre V2 Handheld refractometer), pH_{NIST} (temperature compensated; HANNA bench top meter pH/ORP 115 v pH21-01) and TCO_2 (mmol L^{-1}; Ciba Corning TCO_2 Analyzer 965, Olympic Analytical, UK) were measured and recorded. The TCO_2 analyzer was calibrated with 2 g L^{-1} CO_2 standard prior to measurements. Aquamedic pH probes were calibrated twice weekly with NIST certified pH buffer solutions and CO_2 gas flow into the header tank was adjusted

accordingly. Seawater samples were also analysed for phosphate and silicate levels according to Nickell *et al.* (2003).

Seawater quality in randomly selected individual urchin containers was assessed every 2-3 days using Nutrafin Aquarium test kits. Ammonia, nitrite and nitrates were maintained well below 0.4, 0.2 and 5 mg L respectively by a combination of biological filtration, protein skimming and partial seawater exchanges (approximately 5-15% every 2-3 days) to prevent toxicity from metabolic by-products. A 12:12h light dark cycle was maintained throughout.

Physiological Measurements

The urchins used in the current study were reared in the same incubation system for a further 16 months (in addition to the previous 24 months; Suckling *et al.* 2015) before being used for trials to measure the energetics of feeding and growth in September and November. This coincided with the summer period when energy is partitioned towards maturing gonads in the wild (Brockington & Peck 2001). For each feeding trial, nine or ten *S. neumayeri* were chosen randomly from each treatment. Within each microcosm, specimens were separated by placing them in individually labelled 300 cm^3 containers. Each container had a coarse mesh lid that allowed free exchange of water within each microcosm, but retained the urchin and any food or faeces. To measure individual energy budgets, energy absorbed from food was calculated from the quantity of food consumed and the organic mass of faeces produced. The energy lost through maintenance and food processing was calculated from measurements of oxygen consumption and ammonia and urea (nitrogenous waste) production, both before and six days after feeding.

For the 40 month incubation period, *S. neumayeri* were fed every two weeks (Suckling *et al.* 2015) but trials showed that faecal production, and elevated waste production, continued for longer than two weeks, up to 18 days (pers obs). To ensure a full food processing cycle was measured during the experimental period (September to November), from August, *S. neumayeri* were therefore fed every 3 weeks. *S. neumayeri* were fed individually with an excess of fish fillets, *Polachius virens*, (0.48 ± 0.03 g wet mass, 4% of mean wet body mass) and allowed to feed for 48 hours before uneaten food was collected and weighed. A high protein diet is representative of the broad diet in the field whilst importantly providing an easily quantifiable ration. The water uptake, and concomitant increase in weight of uneaten food, was measured through trials in the same microcosms. Faeces were collected every 2 days and dried until faecal production had stopped. Total faecal dry mass (dried at 60 °C to constant mass) and ash free dry mass (AFDM), calculated by subtraction following ignition for 24 hours at 475 °C, were then determined.

To measure routine and feeding respiratory costs, oxygen consumption was measured before and 6 days post-feeding (defined as pre and post feeding) using closed cell respirometry (following, Obermüller *et al.* (2010). The night before experiments *S. neumayeri* were transferred from their individual containers into respirometers with mesh lids. Before they were closed, respirometers were flushed with seawater from the experimental system, ensuring that any faeces were removed. Respirometers were matched to the size of *S. neumayeri* so that a 10-20% reduction in oxygen was recorded in 3-5 hours, and experiments were stopped before oxygen concentration fell below 80% of saturation values. Oxygen concentration was measured using a Fibox-3 fibre optic oxygen sensor using an individually calibrated oxygen sensitive foil glued into each respirometer (Morley *et al.* 2007). Two or

three blanks were run simultaneously to measure background changes in oxygen concentration. The volume of each urchin was measured using Archimedes principle and the volume of water in each respirometer calculated by subtraction.

At the end of respirometry measurement, the energy lost through nitrogenous waste production was estimated by measuring ammonia and urea in the water in each respirometer. Ammonia concentration in the chamber water was measured with a Turner Designs TD-700 fluorometer, fitted with a near UV mercury vapour lamp and a 310-390 nm excitation filter, following the ortho-phthaldialdehyde (OPA) method of (Holmes *et al.* 1999). Samples were analysed in triplicate and calibration was by standard dilution (four concentrations in triplicate). The remaining seawater was frozen at -80 °C and urea concentration was measured with a Lachat Quikchem 8500 flow injection auto-analyser at the Scottish Association for Marine Science using the Lachat Method 10-206-00-1-A for determination of urea in waters by flow injection analysis colorimetry. However, urea concentration in samples was not significantly different from background levels (blank) in 12 of the 16 treatment-month-feeding combinations (Z-tests) confirming that *S. neumayeri* is largely ammonotelic. Urea production was, therefore, excluded from further analysis.

The results from these measurements of oxygen consumption and nitrogen production were used to calculate the atomic O:N ratio. O:N ratios vary from around 3 for protein only catabolism to over 100 for diets dominated by lipids and carbohydrates (Mayzaud & Conover 1988). The change in O:N ratio before and after feeding therefore indicates how metabolic substrate use varied through the period of feeding.

Calculation of energy budget

Energy available for growth was assessed by converting the physiological measurements into energy equivalents, expressed in J individual^{-1} h^{-1}. The energy budget modified from Winberg (1960) partitioned the energy consumed from food (C) into: respiratory costs (R), waste production (U) as ammonia or faeces (F) and the scope for growth (SfG):

$$C = R + U + F + SfG$$

The energy of the consumed food (C) was calculated using the supplier's (Waitrose) nutritional information. Each 100 g (wet mass) of food contained 340 kJ of energy which was largely in the form of protein (19.3 g of protein, 0.3 g of fat and 0 g of carbohydrate).

The time course of SDA has been calculated for several Antarctic marine invertebrates (2 to 13 days; Peck 1998; Robertson *et al.* 2001; Peck *et al.* 2008) but not *S. neumayeri*, so data from another marine invertebrate, which also has a largely protein based metabolism, *Nacella concinna*, was used (Fraser, Clarke & Peck 2002). The peak of SDA of *N. concinna* at 0°C occurred between days 5 to 7 and so the oxygen consumption on day 6 was calculated to be 1.6 times the average daily elevation in oxygen consumption through the duration of the SDA (Peck & Veal 2001). Therefore, to estimate the respiratory cost of processing food through the whole *S. neumayeri* SDA, the value for the peak SDA, measured at 6 days post feeding

248 was divided by 1.6 to estimate the average daily increase in standard metabolic rate and
249 nitrogen waste production.

250

251 As the food was largely protein and nitrogenous waste production of *S. neumayeri* is
252 predominantly in the form of ammonia (Brockington & Peck 2001), a literature value of
253 0.484 J $\mu molO_2^{-1}$ was used to convert oxygen consumption into an energy cost (Elliott &
254 Davison 1975). The energy loss through ammonia (U) were also calculated using literature
255 energy conversion factors of 0.348 J μmol^{-1} (Elliott & Davison 1975).

256

257 Absorbed energy (A) was calculated from the proportion of the consumed AFDM (M_C) that
258 was retained and not egested as faecal AFDM (M_F):

259

260 $A = ((M_C - M_F)/ M_C) * C$

261

262 The scope for growth was calculated as:

263 $SfG = A - (R + U)$

264

265 **Growth**

266 At the end of experiments in both September and November, *S. neumayeri* volume was
267 measured, urchins were then dissected and wet mass, dry mass and AFDM of gonad (G) and

the rest (S; mainly skeleton) of each animal were measured. Measurement of dry and ash mass followed the same protocol as described above for faeces. From these masses Gonad Somatic Index (%) was calculated as:

$$GSI = G/(G+S) \times 100$$

Prior to drying, a small piece of gonad was weighed, dried and the total carbon, hydrogen and nitrogen contents were measured in a CHN analyser Model CE 440 (Exeter Analytical, Inc., Massachusetts, USA). Each run was calibrated with acetanilide standards. From the CHN data C:N and C:H ratios were calculated.

Statistics

Data were tested for normality with Anderson-Darling tests. Non-normal data were box cox transformed to achieve normality before the fixed effect of treatment and the random effects (to account for repeated measures) of both feeding and month were tested with ANOVA. When a factor had a significant effect, *post hoc* Tukey tests were used. When a factor was still non-normally distributed, even after transformation, differences were analysed using non-parametric Kruskal Wallis tests.

Results

Water chemistry

289 In each system, once treatment conditions had been reached, water chemistry in the urchin
290 tanks was very stable through the 40 month duration of experiments (Table 1).

291

292 **Energetics**

293 *S. neumayeri* consumed more than twice as much food (105% more) in November than
294 September (ANOVA: $F_{(1,64)} = 35.7$, $P < 0.01$) and in both months more food was consumed
295 in the low temperature control than pH treatments (20-30% more consumed; ANOVA, $F_{(3,64)}$
296 = 6.6, $P < 0.01$; Tukey tests, *pH -0.3*, $T = 3.7$ and *pH, -0.5* $T = 4.1$, $P < 0.01$; Table 2). The
297 absorption efficiency of organic matter from food was also lower in September than
298 November ($F_{(1,64)} = 40.8$, $P > 0.01$; Table 2). More energy was therefore absorbed (A) from
299 food in November than September 2012 ($F_{(1,64)} = 35.0$, $P < 0.01$; Fig. 1) and low temperature
300 control individuals absorbed significantly more energy than both pH treatments ($F_{(3,64)} = 5.8$,
301 $P < 0.01$; *pH -0.3*, $T = 3.5$ and *pH -0.5*, $T = 3.7$, $P < 0.01$).

302

303 There was no significant difference in oxygen consumption, between months ($F_{(1,139)} = 1.2$, P
304 = 0.27; Fig. 2) but there was a significant difference between treatments ($F_{(3,139)} = 3.8$, P =
305 0.01). The interaction between month and treatment was just non-significant ($F_{(3,139)} = 2.6$, P
306 = 0.06), so overall, lower oxygen consumption was observed in the low temperature control
307 compared to the high temperature control ($T = 2.6$, $P < 0.05$) and the -0.3 pH treatment (T =
308 3.0, $P < 0.05$). Metabolic rate increased post feeding ($F_{(1,139)} = 6.3$, $P = 0.01$), resulting in an
309 increase in energy costs as food was processed and assimilated.

310

311 Ammonia excretion increased post feeding (Kruskal Wallis test: $H = 12.7$, $P < 0.01$; Fig. 3)
312 but there was no significant difference in the magnitude of this increase between months ($H =$
313 0.1, $P = 0.8$; Fig. 1c) or treatments ($H = 2.2$, $P = 0.54$). The O:N ratio was generally between
314 2 and 4, indicating that the metabolic substrate was almost exclusively protein (Fig. 4). There
315 was no effect of treatment ($H = 6.7$, $P = 0.08$) or month ($H = 1.0$, $P = 0.31$). There was also
316 no significant difference in the change in O:N ratio post feeding between months ($H = 0.0$, P
317 $= 0.94$; Fig. 4) or between treatments ($H = 2.4$, $P = 0.49$).

318

319 Whilst the scope for growth (SfG) was significantly lower in September than November ($H =$
320 15.5, $P < 0.01$; Fig. 4) there was no significant difference between treatments ($H = 5.3$, $P =$
321 0.15), although the general trend mirrored that of energy gain from food.

322

323 **Composition**

324 There was no significant difference in the organic mass (AFDM) of test (month, $F_{(1,69)} = 1.9$,
325 $P = 0.18$; treatment $F_{(3,69)} = 2.2$, $P = 0.10$), gonad (month, $F_{(1,69)} = 0.2$, $P = 0.70$; treatment
326 $F_{(3,69)} = 1.2$, $P = 0.32$), gonad somatic index (month, $F_{(1,69)} = 1.0$, $P = 0.33$; treatment $F_{(3,69)} =$
327 0.7, $P = 0.57$) or gonad C:N ratio between months or between treatments (Table 3). There
328 was a small, but significant difference in gonad C:H ratio between treatments ($H = 14.4$, $P <$
329 0.01) but not between months ($H = 0.35$, $P = 0.55$). Gonads in the low temperature control
330 had the lowest C:H ratio compared to higher temperatures.

331

332 ## Discussion

Growth and energetics

This study describes the longest incubation to date of an Antarctic marine invertebrate to the combined stressors of temperature and ocean acidification and significantly extends the published time series (Suckling *et al.* 2015). After forty months exposure, there was little effect of the treatment conditions on adult *Sterechinus neumayeri* somatic and reproductive tissue mass, elemental composition or scope for growth. However there was a significant effect on oxygen consumption and energetics, with lower metabolic rates and energy absorption in the individuals subjected to elevated temperature. *S. neumayeri* held at +2°C had an elevated metabolic rate, as expected, due to the rate increasing effect that temperature has on biochemical reactions (Clarke 1983; Hochachka & Somero 2002). Indeed the data are very similar to our previous study where metabolic rates of *S. neumayeri* was initially elevated in response to incubation at +2°C with combined OA stressors, but any difference became non-significant after 8 months of incubation (Suckling *et al.* 2015). Average metabolic rates after 40 months at 2 °C were between 2 and 3 μmol O_2 h^{-1} g $AFDM^{-1}$ which is slightly above summer values measured in the wild (Brockington & Peck 2001), where temperatures are above zero, but rarely reach 2°C (Venables, Clarke & Meredith 2013). What is surprising, however, is that the animals at high temperature consumed less food and also absorbed less energy (Fig. 1,Table 2). With their elevated metabolic rates, compared to the animals kept at 0°C, they would be expected to consume more food to fuel their elevated metabolism, which was clearly not the case for *S. neumayeri*. The effects of temperature on feeding rate and energy absorption vary between urchin species (Hill & Lawrence 2006; Zhao *et al.* 2015). For example, *Strongylocentrotus intermedius* consumed less food at higher temperatures which led to a reduction in gonad production (Zhao *et al.* 2015). Under increased warming, the metabolic rates of many ectotherms are expected to increase at greater rates than consumption which

could in turn lead to a reduction in ingestion efficiency, ultimately resulting in energy deficits (Lemoine & Burkepile 2012). How an increase in temperature will effect energy budgets will depend on the thermal reaction norms of biochemical pathways and the proximity of the elevated temperature to the upper boundary of their thermal window (Angilletta 2009). After 40 months in this study, there was, however, no significant difference in animal size, reproductive allocation, or skeletal mass between the different treatments and all individuals were still burning protein as their main food source (Fig. 4). *S.neumayeri,* of the size used here (with test diameters above 20mm), grow very slowly, are difficult to age (Brey *et al.* 1995; Brockington & Peck 2001) and therefore any difference in growth rate may be difficult to detect. It has been estimated that in *S. neumayeri* only 5% of food is allocated to growth, with the remaining 95% going towards reproduction (Brey *et al.* 1995), thus any reduction in nutrition would be expected to affect reproduction first. However, more subtle effect of temperature may lead to differences in energy allocation, some of which may have been missed in the current study.

Two recent studies on echinoderms, albeit on larvae, demonstrated the potential effects of altered pH on the digestive system; with smaller stomachs and reduced feeding performance in the sand dollar *Dendraster excentricus* (Chan, Gruenbaum & O'Donnell 2011) and larger stomachs and increased energetic requirements in the urchin *Stronglycentrotus droebachiensis* (Dorey et al. 2013). The importance of feeding and food processing has also been demonstrated in adult urchins. Individuals that were feeding were able to partially compensate extracellular pH while individuals with empty digestive systems were suffering severe metabolic acidosis (Stumpp *et al.* 2012). With reported effects of ocean acidification on energy allocation (Pan,

Applebaum & Manahan 2015) and feeding behaviour (Barry *et al.* 2014) an increasing number of studies are reporting an interaction between OA stressors and nutritional status (Sandjensen & Pedersen 1994; Melzner *et al.* 2011; Pan, Applebaum & Manahan 2015). Hence there is *a priori* evidence that altered environmental conditions, especially low pH, can affect the energetics of food processing. Which mechanism is most likely to underlie the physiological effects of treatment, particularly the effect of temperature, is impossible to determine without further study.

Bigger differences were found in this study between the two sample months, September and November, than between treatments. In November, consumption of food and absorption of energy were higher, leading to a higher SfG in all treatments. November is the start of the austral summer, the time of peak spawning of *S. neumeyeri* on the WAP (Pörtner, Bock & Reipschlager 2000) and when spawning in the laboratory was most successful (Suckling *et al.* 2015). The presence of seasonal cycle, in spite of *S. neumayeri* being kept in constant temperature and photoperiod conditions for more than 40 months shows that these endogenous rhythms are deeply entrained within this species.

Implications for the benthic ecosystem

This long term study has shown that the Antarctic sea urchin, *Sterechinus neumayeri*, is relatively robust to the effects of near future ocean acidification. The results of the current study show that temperature had a greater effect on the acclimated physiology of *S. neumayeri* than low seawater pH, although there was an indication of an interactive effect, as is being found in

404 an increasing number of studies of marine ectotherms (Schram *et al.* 2014; Feidantsis *et al.*
405 2015). Recent studies have found that some echinoid taxa have a relatively high capacity to
406 buffer the pH of internal fluids against OA stressors (Sandjensen & Pedersen 1994; Stumpp *et*
407 *al.* 2012; Collard *et al.* 2015). This appears to be in part due to their ability to accumulate
408 bicarbonate in the coelomic fluid to reduce the impact of acidosis (Stumpp *et al.* 2012). *S.*
409 *droebachiensis* studied by Stumpp et al. (2012) live in a region that has high seasonal variation
410 in seawater pCO_2 and organism physiological plasticity and resilience are expected to correlate
411 with experienced environmental variation (Gaston *et al.* 2009). The Western Antarctic
412 Peninsula has a stable thermal environment (Venables, Clarke & Meredith 2013) but large
413 variations in pH have been recorded in shallow coastal waters, between pH 7.6 and 8.3 (Bjork
414 *et al.* 2014; Collard *et al.* 2015) which may be correlated with the capacity of *S. neumayeri* to
415 cope with changes in ocean acidification whilst being more sensitive to small changes in
416 temperature.

417

418 The focus of recent laboratory studies towards longer term ocean acidification incubations,
419 particularly for cold water species that have incubated adults for a full reproductive cycle and
420 across multiple generations is providing us with a clearer picture of the capacity of echinoderms
421 to cope with predicted future environmental conditions (Stumpp *et al.* 2012; Dupont *et al.*
422 2013; Suckling *et al.* 2015). As more detailed environmental manipulations are conducted, it
423 is becoming apparent that the subtlety of response is increasingly complex (Munday *et al.* 2009;
424 Kroeker, Micheli & Gambi 2013; Heuer & Grosell 2014). The differences in food consumption
425 and energetics of food processing found in *S. neumayeri*, in the current study, require further
426 studies that combine different ration sizes along with multiple environmental stressors, in order
427 to disentangle the mismatch between food consumption and the energetics of food processing.

428 However, studies to date show that *S. neumayeri* is robust to the impact of near future ocean
429 acidification and may actually benefit from a small rise in environmental temperature (Table
430 4). As *S. neumayeri* are an abundant, keystone, Southern Ocean species, at depths shallower
431 than 20 m, any change in food consumption or conversion efficiency of energy into body
432 tissues could cause a major shift in energy flow through the shallow water ecosystem.

433

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441

442

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Figure Legends

Figure 1. The energy absorbed from food. All values are in Joules per hour per g ash free dry mass. Treatments are: *Low temperature control* = -0.3 °C, pH 8.0; *High temperature control* = 2 °C, pH 8.0; *pH -0.3* = 2 °C, pH 7.8; *pH -0.5* = 2 °C, pH 7.5. Filled bars = September, open bars = November. ** indicates a significant difference in the energy absorbed between months ($F_{(1,64)} = 35.0$, $P < 0.01$). Different letters indicate that low temperature controls absorbed significantly less energy than other treatments (ANOVA $F_{(3,64)} = 5.8$, $P < 0.01$; Tukey tests, *pH -0.3*, $T = 3.5$ and *pH -0.5*, $T = 3.7$, $P < 0.01$). Mean (±SE).

Figure 2. Oxygen consumption of *S. neumayeri*, pre and 6 days post feeding, in September and November. Treatments are: *Low temperature control* = -0.3°C, pH 8.0; *High temperature control* = 2 °C, pH 8.0; *pH -0.3* = 2 °C, pH 7.8; *pH -0.5* = 2 °C, pH 7.5. A, indicates that low temperature controls consumed less oxygen than high temperature control and *pH -0.3* treatments (ANOVA, $F_{(3,139)} = 3.8$, P = 0.01; High temperature control, $T = 2.6$, $P < 0.05$; *pH -0.3* treatment, $T = 3.0$, $P < 0.05$). * indicates that there was a significant increase in oxygen consumption post feeding (ANOVA, $F_{(1,139)} = 6.3$, $P < 0.05$). Mean (±SE).

Figure 3. Ammonia production of *S. neumayeri* in September and November before and 6 days after feeding. Treatments are: *Low temperature control* = -0.3 °C, pH 8.0; *High temperature control* = 2 °C, pH 8.0; *pH -0.3* = 2 °C, pH 7.8; *pH -0.5* = 2 °C, pH 7.5. ** indicates a significant difference between pre and post feeding (Kruskal Wallis test: $H = 12.7$, $P < 0.01$).

654 Fig. 4. Atomic O:N ratio for *S. neumayeri* in September (top panel) and November (bottom
655 panel) before and 6 days after feeding. Treatments are: Tcur = -0.3 °C, pH 7.8; pHcur = 2 °C,
656 pH 8.1; pH-0.3 = 2 °C, pH 7.8; pH-0.5 = 2 °C, pH 7.5. * indicates a significant difference
657 between pre and post feeding.
658

659 Figure 5. The scope for growth in September (filled bars) and November (open bars). **
660 indicates a significant difference between months ($H = 5.3$, $P < 0.01$). *Low temperature*
661 *control* = -0.3 °C, pH 8.0; *High temperature control* = 2 °C, pH 8.0; *pH -0.3* = 2 °C, pH 7.8;
662 *pH -0.5* = 2 °C, pH 7.5. Mean (±SE).

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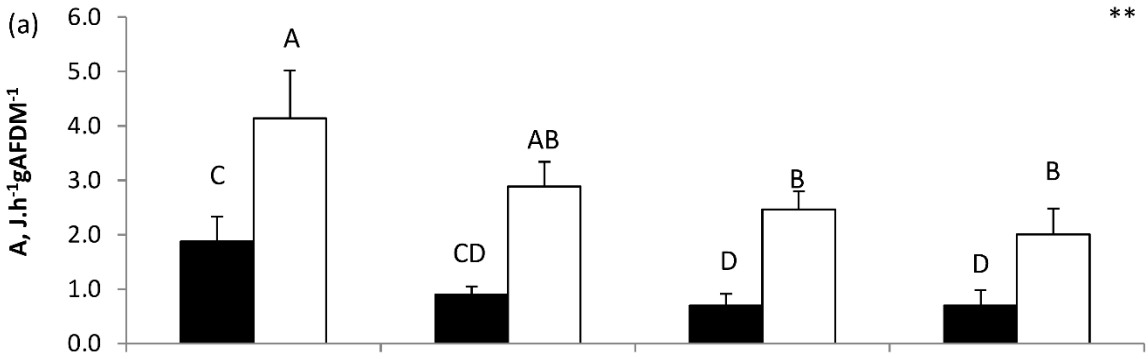

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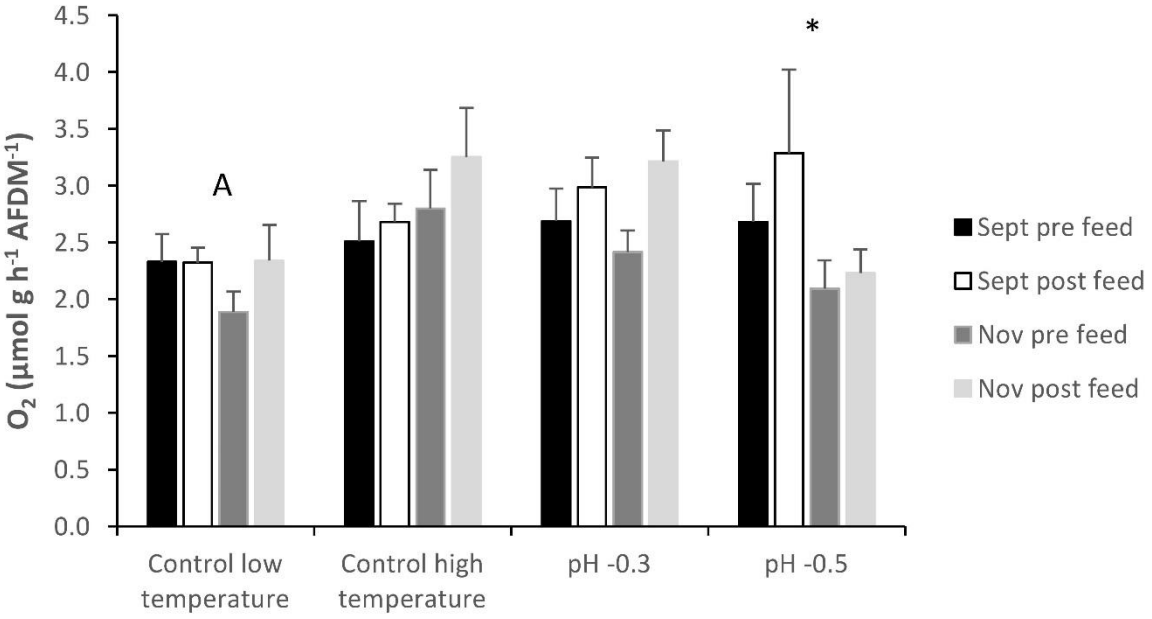
694 Fig. 1



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696 Fig. 2
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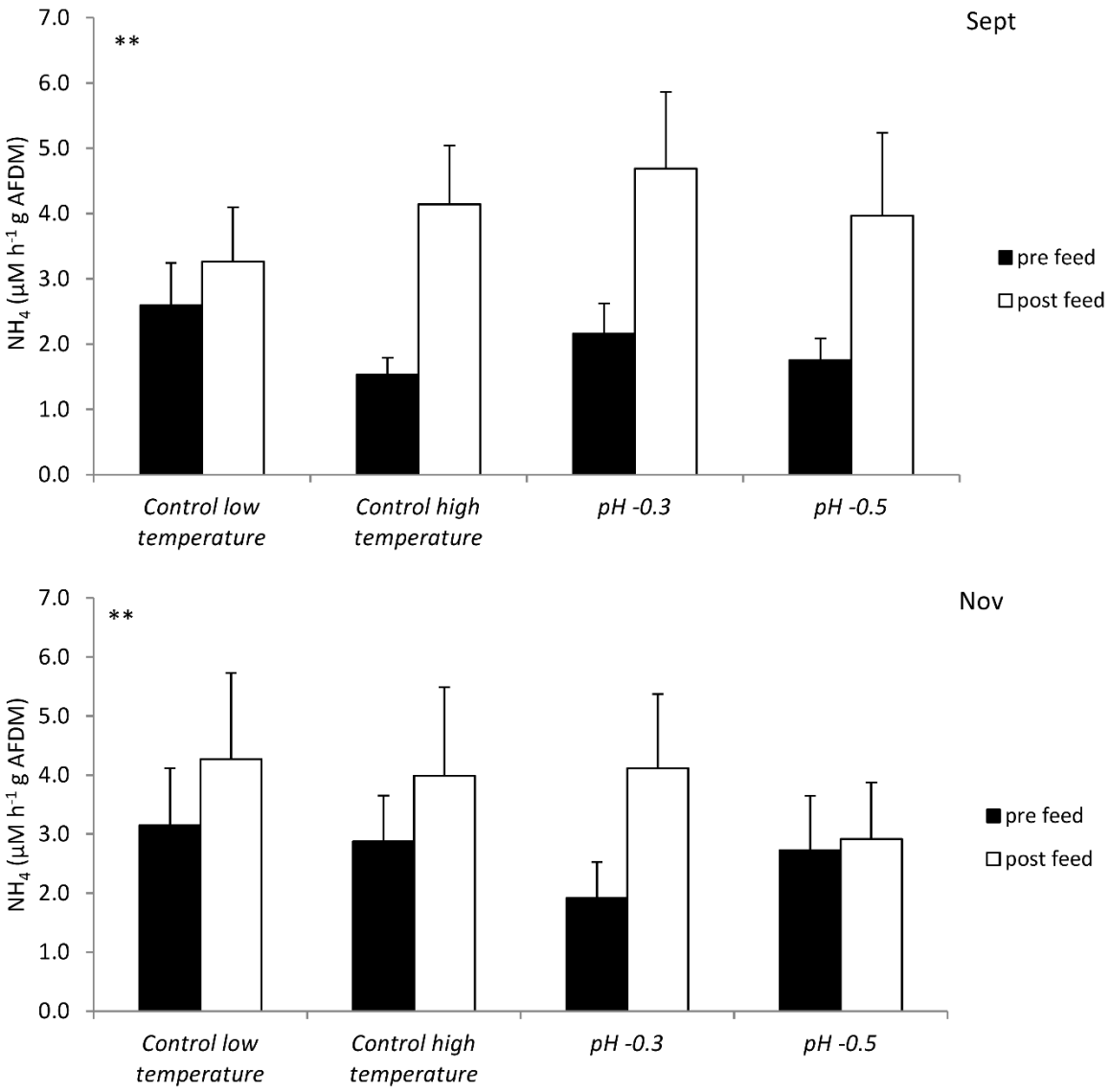

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699 Fig. 3.

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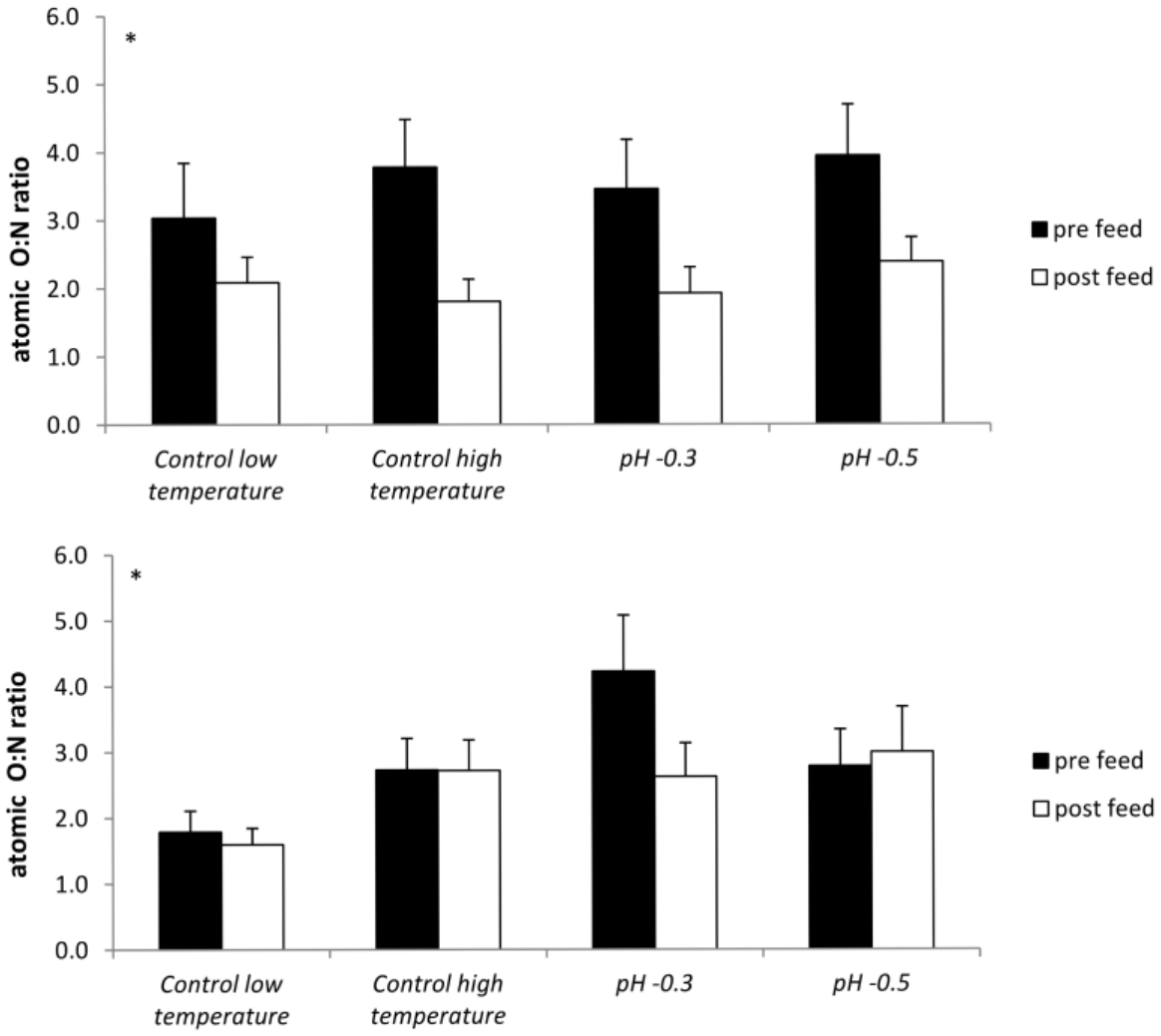
6.0
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atomic O:N ratio
*
Control low temperature
Control high temperature
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pH -0.5
pre feed
post feed
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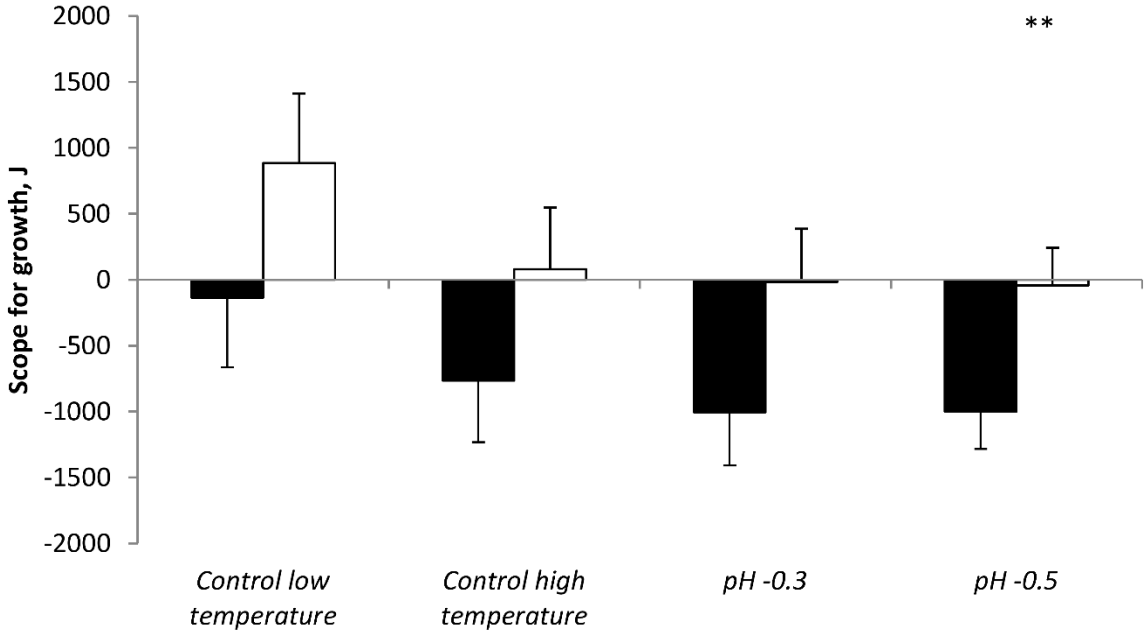

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706 Fig. 5

Seawater parameter	Low temperature control	High temperature Control	-0.3 pH	-0.5 pH
Alkalinity	1733 ± 25	1851 ± 37	1753 ± 40	1805 ± 34
pCO_2 (µatm)	417 ± 15	420 ± 13	834 ± 39	1361 ± 36
pH $_{NIST}$	7.98 ± 0.02	8.00 ± 0.01	7.72 ± 0.01	7.52 ± 0.01
Ω calcite	1.20 ± 0.10	1.50 ± 0.03	0.76 ± 0.02	0.51 ± 0.02
Ω aragonite	0.75 ± 0.06	0.9 ± 0.02	0.48 ± 0.01	0.32 ± 0.01
Temperature (°C)	-0.3 ± 0.0	1.7 ± 0.1	1.9 ± 0.1	2.2 ± 0.1
Salinity (psu)	35 ± 0.2	35 ± 0.2	35 ± 0.2	35 ± 0.1

Table 1: Mean (± SE) water parameters in the adult *Sterechinus neumayeri* microcosm over the course of the experiment following the format of Barry *et al.*, (2010). Values for pCO_2, Ω calcite, Ω aragonite and total alkalinity were modelled from CO2SYS (Lewis & Wallace 1988) with refitted constants (Mehrbach *et al.* 1973; Dickson & Millero 1987).

Parameter		*Low temperature control*	*High temperature control*	*pH -0.3*	*pH -0.5*
A_{eff}	Sept	0.77 ± 0.06a	0.71 ± 0.05a	0.66 ± 0.06a	0.55 ± 0.08a
	Nov	0.87 ± 0.02b	0.91 ± 0.02b	0.87 ± 0.02b	0.91 ± 0.03b
C	Sept	2.4 ± 0.5a	1.3 ± 0.2ab	1.0 ± 0.3b	1.0 ± 0.2b
	Nov	4.8 ± 1.1c	3.1 ± 0.5cd	2.7 ± 0.3d	2.2 ± 0.5d

Table 2: Absorption efficiency (A_{eff}) and energy consumed (C, $J.h^{-1}gAFDM^{-1}$), in September and November. Treatments are: *Low temperature control* = -0.3 °C, pH 8.0; *High temperature control* = 1.7 °C, pH 8.0; *pH -0.3* = 1.9 °C, pH 7.8; *pH -0.5* = 2.2 °C, pH 7.5. Mean ± SE. Different lower case letters indicate that absorption efficiency was lower in September than November ($F_{(1,64)} = 40.8$, $P > 0.01$). Different lower case letters indicate that more energy was consumed in the low temperature control than other treatments (ANOVA, $F_{(3,64)} = 6.6$, $P < 0.01$; Tukey tests, *pH -0.3*, $T = 3.7$ and *pH -0.5*, $T = 4.1$, $P < 0.01$) and was less in September than November ($F_{(1,64)} = 35.7$, $P < 0.01$).

Parameter		*Low temperature control*	*High temperature control*	*pH -0.3*	*pH -0.5*
Test AFDM, mg	Sept	734 ± 56	611 ± 72	628 ± 89	549 ± 64
	Nov	791 ± 105	712 ± 80	703 ± 50	600 ± 68
Gonad AFDM, mg	Sept	629, 510-859	634, 358-675	506, 337-746	685, 288-828
	Nov	728, 407-935	511, 362-871	554, 349-675	589, 345-777
GSI	Sept	48.1 ± 1.6	46.2 ± 2.5	46.2 ± 2.3	48.9 ± 3.7
	Nov	47.2 ± 4.3	42.4 ± 3.7	43.8 ± 3.2	47.1 ± 3.7
C:N	Sept	5.4, 5.1-5.9	5.7, 5.3-6.0	5.6, 5.2-6.0	6.0, 5.3-6.3
	Nov	5.9, 5.5-6.4	6.1, 5.4-6.2	5.8, 4.7-5.8	6.4, 5.3-8.0
C:H	Sept	0.52, 0.51-0.53a	0.53, 0.53-0.54ab	0.53, 0.52-0.54bc	0.54, 0.53-0.54c

Nov	0.53, 0.52-0.53a	0.53, 0.52-0.53ab	0.54, 0.54-0.55bc	0.54, 0.53-0.55c

Table 3. The ash free dry mass (AFDM) of the test and gonad, the gonad somatic index (GSI), the carbon to nitrogen (C:N) and carbon to hydrogen (C:H) ratio in the gonad in September and November. Values are means ±SE or median, interquartile range (the latter is used where data were not normally distributed, even after transformation. Different letters after the interquartile range indicate significantly different C:H ratios.

Duration of incubation	Trait	Control low temperature	Control high temperature	pH -0.3	pH -0.5	Ref
6 months	Egg size	+	-	-(-)	-	Suckling et al. 2015
	Fertilization success	=	+	=	=	Suckling et al. 2015
	Hatching success	+			-	Suckling et al. 2015
	Larval survival	+	+		-	Suckling et al. 2015
17 months	Egg size	-	+(-)	+	+(+)	Suckling et al. 2015
	Fertilization success		+		-	Suckling et al. 2015
	Hatching success	=	=	=	=	Suckling et al. 2015
	Larval survival	-	+		-	Suckling et al. 2015
8 to 24 months	Metabolic rate		=	=	=	Suckling et al. 2015
8 to 40 months	Test growth	=	=	=	=	Suckling et al. 2015 Current study
8 to 40 months	Gonad allocation	=	=	=	=	Suckling et al. 2015 Current Study
After 40 months	Metabolic rate	-	+	+	+	Current Study
After 40 months	Food consumption	+	-	-	-	Current Study
After 40 months	Ammonia production	=	=	=	=	Current Study
After 40 months	Scope for growth	=	=	=	=	Current Study

Table 4. Summary of effect of combined temperature and pH treatments on *S. neumayeri*