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Gross Growth Efficiency of *Daphnia magna*:Genetic Variance and Effects of Temperature

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ABSTRACT

Gross Growth Efficiency (GGE) is defined as the ratio between Somatic Growth Rate (SGR) and food consumption, and hence represents the quantity of food eaten that is converted to body mass. SGR correlates with the intrinsic rate of population increase and GGE may correlate with trophic efficiency. SGR and GGE can provide potential focal traits to link ecology to physiological traits and understanding the genetic and environmental sources of variations and covariations of these traits is important.

I compared SGR and GGE of 10 different clones from a single population of the zooplankton *Daphnia magna* at a single temperature and found significant genetic variation in both traits. This implies that these traits can evolve in a population. However, there was no genetic correlation between SGR and GGE implying that the traits can evolve independently of each other. I also measured SGR and GGE of a single clone at eight different temperatures (range 12 - 28°C) and as expected, SGR increased with increasing temperature, and so did GGE. The generality of this latter result remains to be shown across different clones. I suggest that the observed temperature response for GGE is consistent with expected selective pressures in the wild, where negative correlations between temperature and food abundance throughout the season make it more important to maximize efficiency at high temperatures when food abundance is more likely to be limiting.

INTRODUCTION

Growth plays a crucial role in the life cycle of animals as it connects the age and size in life history transitions (Van Doorslaer and Stoks 2005; Gotthard, 2001). Age and size are fundamental traits that have strong effect on fitness, thus in general, study of juvenile growth may be significant to understanding and explaining life history evolution (Gotthard, 2001). Many previous studies have shown that Somatic Growth Rate (SGR) is positively correlated with the intrinsic rate of population increase (r), primarily due to the effect on the length of the juvenile period (Gotthard, 2001; Lampert and Trubetskova, 1996; Stearns, 1992). Growth rate will be influenced by food intake, but also by the Gross Growth Efficiency (GGE), which is the quantity of food ingested that is converted to body mass (Doupé and Lymbery, 2003). The efficiency of an animal to use available food resources to enhance its growth can therefore have a large influence on its fitness (Robinson and Partridge, 2001). GGE may also have an additional ecological effect by influencing trophic efficiency. If food ingested by animals is poorly converted into body mass, the amount of energy from a lower trophic level to a higher trophic level becomes small (Elser and Urabe 1999; Urabe and Watanabe,1991), hence low GGE is expected to lead to low trophic efficiency. Thus, SGR and GGE provide potential focal traits to link ecology to physiological traits and understanding the genetic and environmental sources of variations and covariations of these traits is important.

For a fixed limiting ration, SGR and GGE are necessarily positively correlated, as increased growth under these conditions can only occur through increased efficiency. Under *ad lib* rations this may change. Genetic variation in feeding rates may lead to a proportional change in SGR, in which case SGR and GGE are not related. Alternatively, higher feeding rates may lead to a more than proportional increase in SGR, causing a positive correlation between SGR and GGE. Finally, higher feeding rates may lead to a less than proportional increase in SGR, causing a negative correlation between SGR and GGE. Knowledge about genetic variance in GGE and genetic correlations between SGR and GGE provides insights into how this trait can be expected to evolve. The following questions can be asked: Is there genetic variance in GGE in natural populations? And if so, will it respond to selection on SGR, such that populations selected for high SGR also have high (or low) GGE?

In addition to the genetic variance, multiple environmental sources of variance in SGR and GGE can be expected. For ectotherms, temperature is one particularly important environmental factor that directly influences their growth physiology (Skoglund *et al.* 2011; Van Doorslaer and Stoks 2005; Heilmayer *et al.,* 2004). Many studies on temperature effect on SGR of ectotherms have been described and show that SGR increase with increasing temperature until an optimum temperature and then rapidly decreased (Masclaux *et al.,* 2009; Giebelhausen and Lampert 2001; Purchase and Brown, 2000; Mitchell and Lampert 2000; Ranta *et al.,* 1993). Results from studies on GGE are either positive or negative. Angilletta and Dunham (2003) and Dawidowicz and Loose

(1992) estimated increase in GGE with increasing temperature which is in contrast with the findings of Berrigan and Charnov (1994) and Atkinson and Sibly (1997), who estimated a negative relationship between GGE and temperature. Thus, it is not clear how we may expect GGE to change with temperature.

In the present study I ask the following questions: How does temperature affect GGE and is there genetic variance in GGE? How are GGE and SGR genetically correlated? To answer the questions, I conducted laboratory studies on *Daphnia magna* to test the effects of temperature on SGR and GGE for a single clone and genetic variation in SGR and GGE among different clones. I also estimated the correlation between SGR and GGE.

Study species

In December 2014, ten genotypes, hereby referred to as clones, of *Daphnia magna* were hatched from ephippia which were collected from a pond at Værøy Island (1.0 ha, 67.687°N 12.672°E). Using a photoperiod of 16L:8D in ADaM medium (Klüttgen *et al.* 1994, SeO2 concentration reduced by 50%), *Daphnia* were cultured at 17°C. *Daphnia* were fed three times a week with Shellfish Diet 1800 (Reed Mariculture Inc, Campbell, CA, USA) at an algae concentration of 4×10^5 cells ml^{-1}. There was weekly exchange of ADaM medium.

Experiment designs

I conducted two experiments, one testing for genetic variation and the other testing the effects of temperature. The first experiment exposed different clones to a single temperature and the second experiment exposed a single clone to different temperatures.

All experimental animals were second clutch juvenile females (≤ 24 hour old) originating from mothers that had been reared for two generations at the experimental temperatures. Based on pilot experiments, feeding regimes during experiments were set to ensure *ad lib* conditions (Table 1).

Table 1: Feeding regimes and duration of growth experiments in *Daphnia magna* for different temperatures

Temperature (°C)	12	15	17	19	22	24	26	28
Duration (days)	9	7	5	5	3	3	3	3
Algae cell concentration (cells ml^{-1})	2.00×10^5	2.38×10^5	2.62×10^5	2.88×10^5	3.24×10^5	3.50×10^5	3.75×10^5	4.00×10^5
Feeding frequency (number of times)	5	4	3	3	2	2	2	2

Somatic Growth Rate (SGR) Method

Juveniles (≤ 24 hour old) were randomly selected from 250ml jars and their start (initial) gut lengths (mm) were measured. Afterwards juveniles were placed individually in 50ml centrifuge tubes containing ADaM medium, fed with Shellfish Diet 1800, and placed in climate cabinets to grow for 3 to 9 days (depending on temperature, Table 1). Following this I measured end (final) gut length. I estimated SGR based on start (initial) gut length and end (final) gut length measurements using microscope photography and IMAGEJ 1.44p software (National Institutes of Health, Bethesda, MD, USA). Dry body mass was calculated from gut length as:

Dry mass (mg)= 0.00679 x gut length$^{2.75}$ (Yashchenko *et al.* 2016).

Somatic growth rate (SGR) was calculated as:

SGR = log (final dry mass (mg)) – log (initial dry mass(mg)) /duration.

Food Consumption Method
Food consumption experiments were done 1 hour after SGR experiments. All equipment used were acclimatized to their experimental temperatures. I transferred spot plates to a water bath after the growth experiments to keep temperature constant. I placed *Daphnia* individually in 3ml spot plate wells to feed and I used spot plate wells without *Daphnia* as reference. I fed individual *Daphnia* with a final algae concentration $3.12x10^5$ cells ml^{-1} for the experiment. After an hour, I took individuals out of the wells so no *Daphnia* was consuming algae. I took 2ml from each spot plate well and mixed this with 8ml isoton in a cuvette before measuring the number of algae (food consumption) left using a Beckman Coulter counter (Beckman Coulter Inc, USA). I did three (3) counts per sample. From this, food consumption was calculated as:

Food consumption (cells/hr) = mean control number of cells (without *Daphnia*) - mean number of cells (with *Daphnia*).

For calculation of GGE, I first adjusted the food consumption of all individuals to a common mean body size (see Statistics). I then used the absolute growth rate (mg dry mass/hour) and divided this by the food consumption (number of algae/hour). Thus, the unit for GGE becomes mg dry mass/algae cell.

Genetic variance of GGE and SGR
I tested for genetic variation in GGE and SGR by using 10 clones at 17°C. Each run consisted of 60 wells, including 50 animals (5 individuals of each clone) and 10 controls. These were distributed into three spot plates, where every clone and some controls were present in each spot plate. Five replicate runs were conducted, giving a total sample size of 250 (10 clones x 25 individuals each).

Effect of Temperature on GGE and SGR
I investigated the effect of temperature on SGR and GGE for a single clone (clone 64) by culturing individuals at eight (8) different temperatures (12 °C, 15°C, 17°C, 19°C, 22°C, 24°C, 26°C and 28°C). I used 25-30 individuals for each temperature. For food consumption, each temperature consisted of 35-40 wells, with 25-30 individuals and 10 controls. These were distributed into two spot plates for each temperature. Total sample size was 235 replicates.

Statistical analyses

All statistical analyses were conducted with Rv.2.9.2. (R Development Core Team). All mixed effect models were implemented using *lmer* function in the package *lme*. I compared different models using AIC and the model with the lowest AIC was chosen as the best model. I controlled for the dry body mass in consumption (as dry body mass varied for the different clones) by adjusting consumption to a common body mass. I also calculated the heritability [(V_g/ V_p), V_g is variation due to genes and V_p is phenotypic variation] of GGE and SGR

I tested the relationship between dry body mass and food consumption for all the clones first using a mixed effect model. The model included consumption as the response variable, dry body mass (the final dry mass after the growth period) as a fixed factor and clone as a random effect. I tested for both random intercepts and random slopes.

Mixed effect model was used to test the genetic variation in GGE. I fitted models where GGE was the response variable and body mass was the fixed effect with clone and plate effect as random effects and tested random intercepts.

Mixed effect model was used to test the genetic variation in SGR. I fitted the model where absolute SGR was the response variable and dry body mass as fixed effect and clone and run effect as random factors.

 I tested for genetic correlation between GGE and absolute SGR using a Pearson correlation test (*cor.test* function). The correlation was done using clonal means of SGR and GGE.

Mixed effect model was used to test the effect of temperature on GGE. I fitted models where GGE was the response variable and body mass and temperature as a continuous variable were the fixed effects and plate as the random effect.

 I tested the effect of temperature on SGR using a linear regression. SGR was fitted as the response variable and body mass and temperature as covariates.

RESULTS

In all clones, food consumption increased as dry body mass increased (Fig. 1) at 17°C. Intercepts differed for all clones but not the slopes (Table 2). A linear model with food consumption (cells/hr) as a response variable and body mass (mg) as an independent variable explained 65% of the variations in food consumption. The overall slope (±SE) for the estimated effect of dry body mass was 110484±3984.
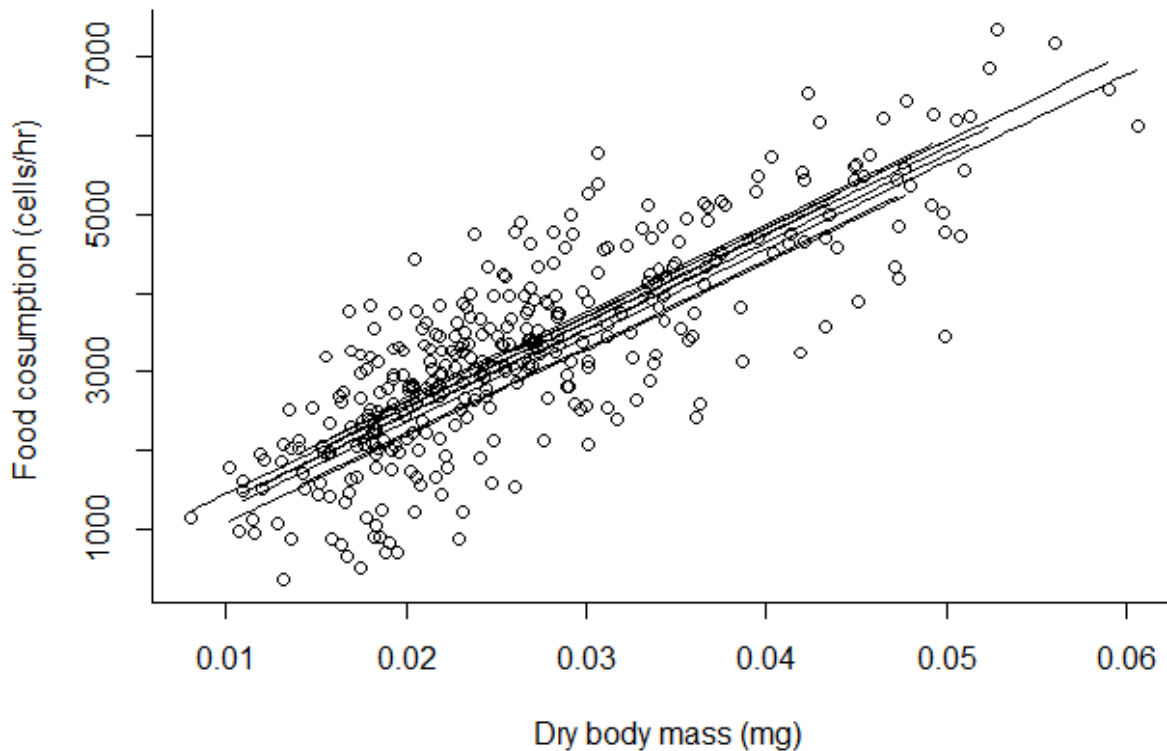

Fig. 1: The relationship between dry body mass and food consumption in 10 clones of *D. magna* at 17°C. Separate regression lines are given for each clone.

GENETIC VARIATION

Somatic Growth Rate (SGR)

Clone had a significant effect on SGR (Table 2). The variance in clone was 1.5×10^{-7} and variance in run was 3.5×10^{-7} and residual was 2.6×10^{-7}. The estimated slope (±SE) of the dry body mass effect was 0.36±0.07. The clone with the highest SGR had a mean value that was 10% larger than the clone with the lowest SGR (Fig. 2). The broad sense heritability of SGR was 0.30

Gross Growth Efficiency (GGE)

The effect of clone could not be removed without a significant increase in AIC (Table 2). The variance in clone was 7.1×10^{-15} and variance in run was 5.6×10^{-14} and residual was 1.8×10^{-13}. The clone with the highest GGE had a mean value that was 16% larger than the clone with the lowest GGE (Fig. 3). The estimated slope ($\pm$SE) of the dry body mass effect was $1.1 \times 10^{-4} \pm 4.0 \times 10^{-6}$. The broad sense heritability of GGE was 0.11.

Table 2: Model selection using AIC for different experiments and traits. Random effects were compared first. Comparing models differing in their fixed effects, the models were fitted with Maximum Likelihood (ML) (REML = F). The models fitted with Restricted Maximum Likelihood (REML=T) compared random effects.

Experiment	Trait	Model		AIC
	Food consumption	Dry body mass + (1|clone) +(1|plate)	Fixed effects (REML= F)	5368.23
		(1|clone) +(1|plate)		5762.65
		Dry body mass + (1 |clone) +(1|plate)	Random effects (REML= T)	5338.28
		Dry body mass + (dry body mass |clone) +(1|plate)		5341.80
		Dry body mass +(1|plate)		5350.19
		Dry body mass + (1|clone)		5406.30
Temperature	GGE	Dry body mass+ Temperature+ (1|plate)	Fixed effects (REML= F)	-5785.83
		Dry body mass* Temperature +(1|plate)		-5780.25
		Dry body mass +(1|plate)		-5773.54
		Temperature+(1|plate)		-5680.76
		(1|plate)	Random effects (REML= T)	-5638.38
		No random		-5574.03

| Genetic variation	GGE	Dry body mass + (1|clone) + (1|plate)	Fixed effect (REML= F)	-8859.42
		(1|clone) + (1|plate)		-8543.25
		Dry body mass + (1|clone) + (1|plate)	Random effects (REML= T)	-8805.47
		Dry body mass + (1|plate)		-8802.35
		Dry body mass + (1|clone)		-8772.08
	SGR	Dry body mass + (1|clone) + (1|run)	Fixed effect (REML= F)	-3331.41
		(1|clone) + (1|run)		-3303.56
		Dry body mass + (1|clone) + (1|run)	Random effects (REML= T)	-3321.81
		Dry body mass + (1|run)		-3307.63
		Dry body mass + (1|clone)		-3293.19
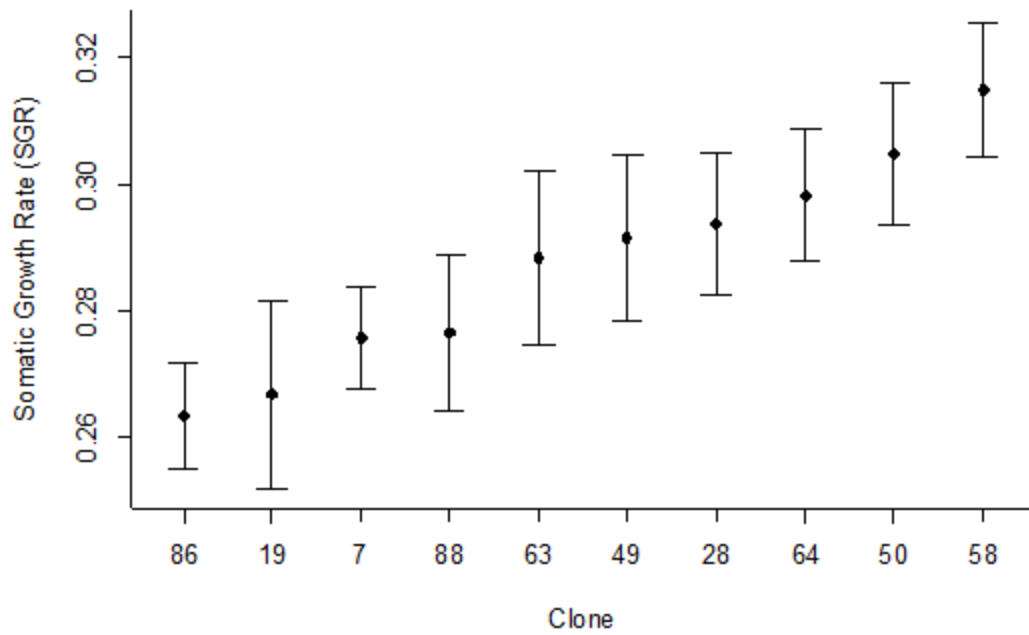
Fig. 2: Somatic Growth Rate (mean±SE) in 10 clones of *D. magna* reared at 17°C.
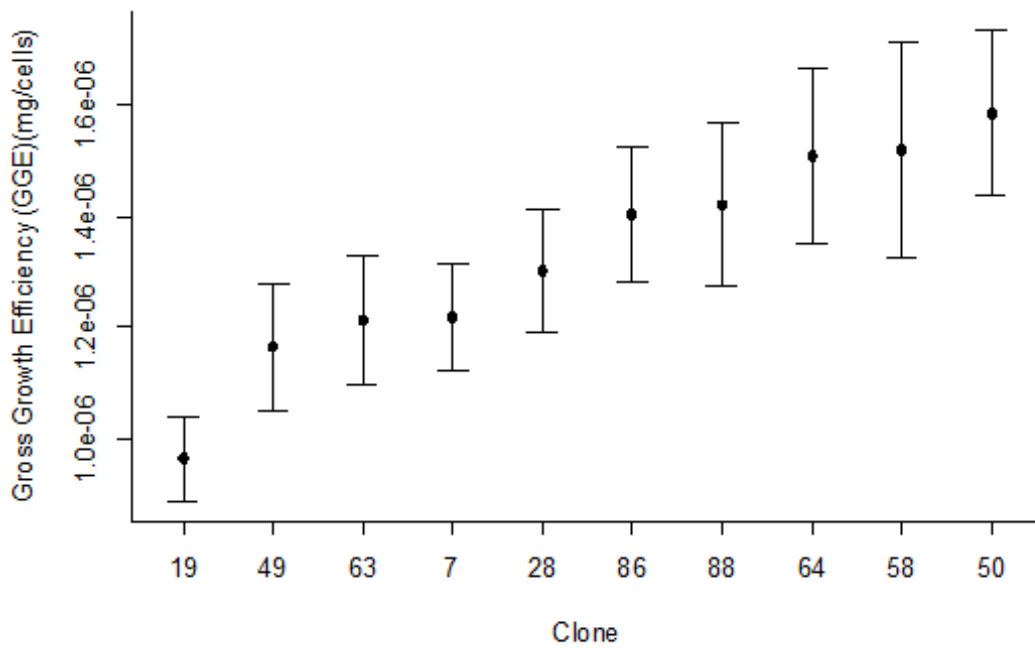

Fig. 3: Gross Growth Efficiency (mean±SE) in 10 clones of *D. magna* reared at 17°C.

EFFECT OF TEMPERATURE

Somatic Growth Rate (SGR)

There was significant variation in SGR across temperatures (Fig 4). SGR increased as temperature increased (r^2=0.90, F test $_{(2, 219)}$ = 1063, p < 2.2e-16). The estimated slope(±SE) of the temperature effect was 0.03±0.0001 (t test $_{(1, 219)}$ =22, p < 2.2e-16). The estimated slope (±SE) of the dry body mass effect was 0.38±0.015 (t test $_{(1, 219)}$ =33, p < 2.2e-16).
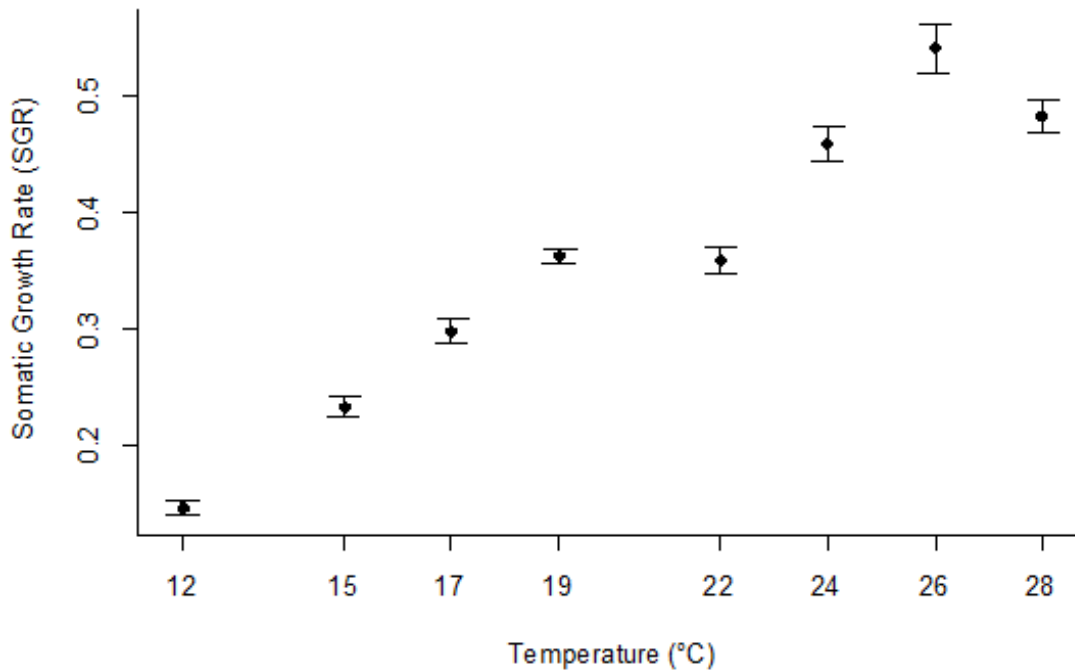
Fig. 4: Effect of temperature on SGR (mean±SE) in a single clone of *D. magna*.

Gross Growth efficiency (GGE)

There was significant variation in GGE across temperatures (Fig. 5, Table 2). The variance in run was 9.4×10^{-14} and residual was 2.3×10^{-13}. The slope (±SE) of the effect of temperature was $6.3 \times 10^{-8} \pm 1.5 \times 10^{-8}$ indicating an increase in GGE with increasing temperature. The estimated slope (±SE) of the dry body mass effect was $1.0 \times 10^{-4} \pm 8.8 \times 10^{-6}$.
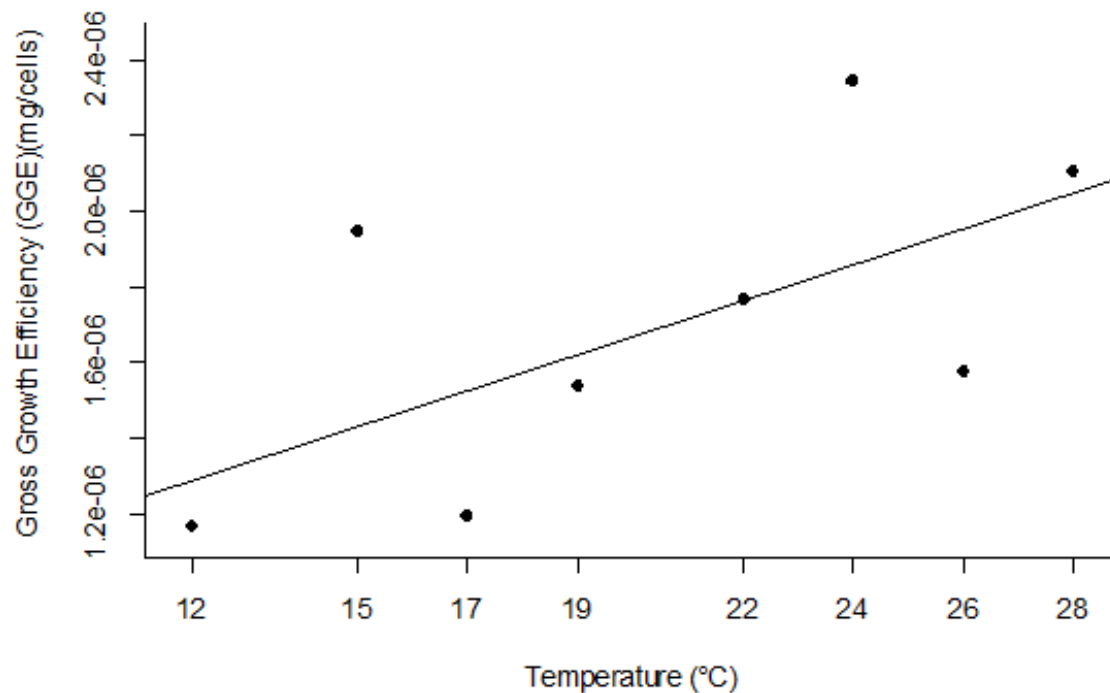
Fig. 5: Effect of temperature on GGE (mg/cells). The points are the mean GGE at each temperature.

CORRELATION

There was no significant genetic correlation between SGR and GGE (Table 3, Fig. 6).

Table 3: Pearson's correlation (95% confidence interval)

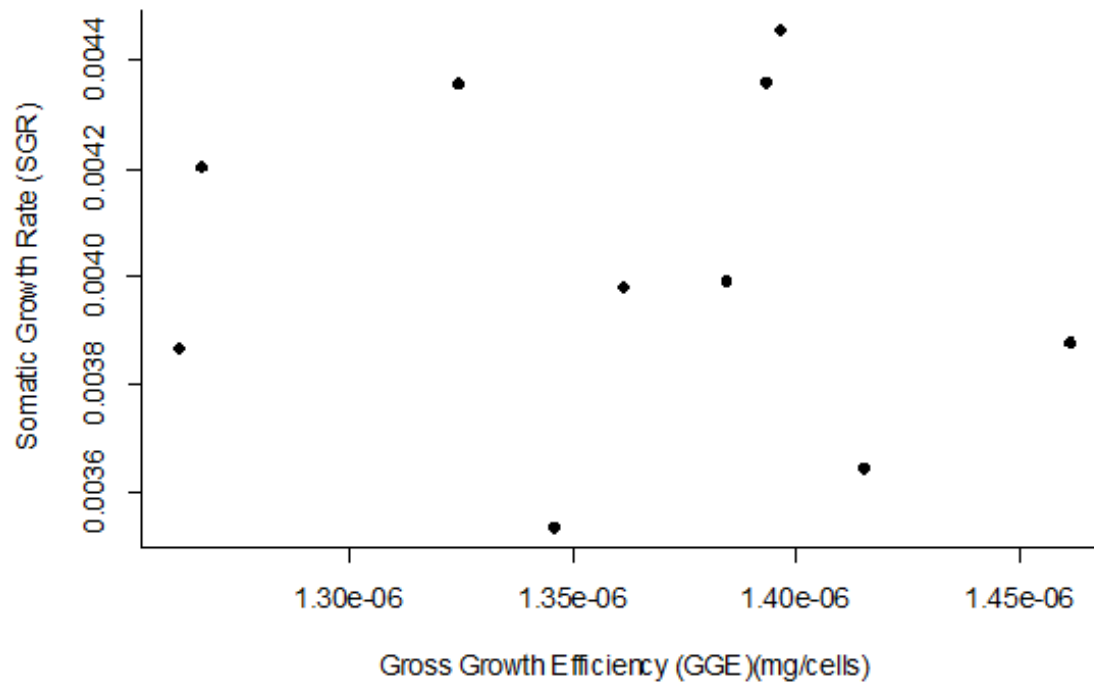|	df	t-test	p-value	95% confidence interval	Correlation
Genetic variation	8	-0.25119	0.808	-0.68 to 0.57	-0.089

Fig. 6: Genetic correlation between absolute SGR and GGE (mg/cells). The points are clonal means of SGR and GGE.

DISCUSSION

In the present study, I tested for variation among clones and effects of temperature on SGR and GGE in *Daphnia magna*. *D. magna* used in the study serve as an important keystone grazer in aquatic ecosystems (Lampert and Kinne, 2011; De Meester *et al.,* 2004; Peters and de Bernardi, 1987).

The current study shows significant genetic variation in SGR among clones, with relatively modest sample size of 22-25 replicates for each clone at $17°$C. Other studies have also found genetic variation in SGR. Rinke and Petzold, (2003) found clonal differences in SGR in *Daphnia* and Thodesen *et al.* (2001, 1999) estimated significant genetic variations in SGR in Atlantic salmon (*Salmo salar*) same as Gjedrem (2000) who estimated genetic variance in growth rate for cold water fish species. Thus, genetic variation in growth rate likely exists in most ectotherms.

In the present study, there is significant genetic variation in GGE with a heritability of 0.11. Some studies also have found genetic variation in GGE in ectotherms, although most of these are from aquaculture species and it is hard to evaluate to what extent they represent genetic variation within natural populations. Gjedrem (2000) concluded that additive genetic variation existed in salmonid species and stated GGE was a heritable trait. Henryon *et al.* (2002) also found significant additive genetic variance in feed efficiency in a farmed population of rainbow trout with heritability of 0.7. In Atlantic salmon, Thodesen *et al.,* (2001) also estimated significant difference in feed efficiency among fill-sib families. Thodesen *et al.* (1999) compared the GGE of offsprings of farmed and wild salmon and estimated a significant variation between the populations. GGE was higher in the farmed salmon than in wild salmon. In contrast, Kinghorn (1983) found no genetic variation in GGE in rainbow trout (*Oncorhynchus mykiss).* The discrepancy between my conclusion and the results obtained by Kinghorn (1983) may be due to the difference in species, but may also be due to differences in methodological approach, as the latter study used oxygen consumption as an indirect measure of food intake.

The present study demonstrates that there is no genetic correlation between SGR and GGE. This means SGR and GGE can evolve independently of each other. Correlation between SGR and GGE may change due to food concentration. Under restricted food ration, SGR and GGE may be necessarily positively correlated, as increased growth under these conditions can only occur through increased efficiency, but under *ad lib* rations this may change (Masclaux *et al.,* 2009; Urabe and Watanabe, 1991; Houde, 1989; Mullin and Brooks, 1970; Beklemishev 1954, 1957,1962). Genetic variation in feeding rates may have led to a proportional change in SGR, in which case SGR and GGE are not related. Contrary to my results, Henryon *et al*., (2002) and Kinghorn (1983) found genetic correlations between feed efficiency and growth rate in rainbow trout. The differences in results may be due to difference in species, but may also be due to differences in methodological approach, as these studies showed genetic correlation among populations, but did not test it within populations as I did.

The present study shows that SGR increases with increasing temperature (12-28 degrees) for a single clone with highest growth rate recorded at $26°$C. Most studies also concluded that somatic

growth increased with increasing temperature until it decreases after reaching the optimum temperature for *D. magna*, zooplanktons and other ectotherms (Lemke and Benke 2004; Dawidowicz and Loose, 1992). Giebelhausen and Lampert (2001) found highest somatic growth at 20°C and then decreases at higher temperatures. Slower somatic growth may be caused by decreasing temperatures which leads reduction in metabolic rates (Angilletta *et al.,* 2004).

There was also a general increase in GGE with increasing temperature. This result is consistent with the findings of Angilletta and Dunham (2003) who found that growth efficiency tends to increase with increasing temperature for most ectotherms suggesting that von Bertalanffy-Perrin model does not apply to majority of ectotherms. The von Bertalanffy-Perrin model predicts that growth rate and growth efficiency should decrease with increasing temperature states. Angilletta and Dunham (2003) observed that only 6 out of 20 species followed von Bertalanffy-Perrin model, indicating that for most species the growth efficiency increase with increasing temperature. At 16°C, GGEs were significantly lower than at higher temperatures for metazooplanktons (Almeda *et al.,* 2011). Sharma and Pant (1984) stated that increase in temperature accelerated ingestion and hence increases growth efficiency in ectotherms. As a potential adaptive explanation for the relationship between temperature and GGE in *Daphnia,* according to Beklemishev (1954, 1957, 1962), in the wild at spring time there is a high concentration of phytoplankton which means there is a high food abundance for zooplankton. During summer with increase in temperature, food availability becomes lower. This means that selection for a high GGE should be strongest during the time of the season when temperatures are highest.

In conclusion, the results show that temperature affects both SGR and GGE. These two traits both increase with increasing temperature (from 12 to 28^{0}C). The results also demonstrate that there is genetic variation in SGR and GGE in ectotherms, which implies that the two traits have the potential to be changed through evolution in populations. The results also show that the traits can evolve independently of each other, as there was no genetic correlation between the two traits. GGE is of ecological importance and further studies on the correlation between GGE and other traits such as age will give a better understanding about selection of this trait.

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APPENDIX

Table 4: Intercepts (Fig. 1) of each clone for food consumption (cells/hr)

Clone	Intercept
7	463.694895
19	258.253282
28	421.338656
49	349.008765
50	152.110383
58	330.115922
63	236.870951
64	409.310474
86	9.529513
88	-23.232842