THE EFFECT OF DIETS ON THE GROWTHRATE OF OYSTER LARVAE/SPAT (*Crossostera gigas*) AT MILE 4, SWAKOPMUND, NAMIBIA

BY:

SOPHIA M. ISALA

STUDENT NUMBER: 200731271

A report submitted to the Department of Fisheries and Aquatic Science, Faculty of Agriculture and Natural Resources, in partial fulfillment of the requirement for the award of the degree of Bachelor of Science(Honours) in Fisheries and Aquatic Science of the University of Namibia.

Supervisor:

MR. A. ESTERHUIZEN

Department of Fisheries and Aquatic Science, Faculty of Agriculture and Natural Resources, University of Namibia

Windhoek, Namibia

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Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. A. Esterhuizen and has not presented elsewhere for the award of the honors degree. All the sources have been duly and appropriately acknowledged.

Candidate Signature: …… Date:

……..

SOPHIA M. ISALA S

(200731271)

Certification

This is to certify that this report has been examined and approved for the award of the honors degree of Bachelor of Science in Fisheries and Aquatic Science of the University of Namibia.

External Examiner……

Internal Examiner……

Head of Department……

Dedication

I dedicate this work to my parents Mr. and Mrs. Isala, my siblings, all relatives and friends as a token of how much I loved doing the course (Fisheries and Aquatic Science) and why they missed me when I was away from home.

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1. INTRODUTION

1.1 General introduction

The pacific oyster *Crassostrea gigas* is an exotic species that was introduced into the west coast estuaries from Japan. Spawning depends on a rise in water temperature of above 18 degree Celsius and only breeds unpredictably in West coast estuaries. Spawning occurs primarily in July and August. Eggs and larvae are planktonic and distributed throughout the water column later they crawl on the bottom searching for suitable habitat before settlement (Alderman, 1974).

The Namibian mariculture industry is focused on the cultivation of oysters and the nutrient- rich Benguella current waters greatly enhance the sustainability for culture. The oyster production at Swakopmund (Beira Aquaculture and Rich Water Oyster Farm) uses a land- based system in which water is pumped from the sea into ponds (man- made) and larvae is reared into tanks within the hatchery. "The Namibian mariculture industry consists primarily of oyster producers and to a lesser extent abalone (*Haliotis midae*). After a year of substantial growth in 2007, the oyster producers suffered severe production losses in 2008, resulting from persistent adverse environmental conditions along the central Namibian coast. The near collapse of the oyster operations in Walvis Bay has resulted in significant restructuring, with some participants leaving the industry". Oellerman and Hitula, (2009)

The highly productive waters along Namibia's central coast present directly associated challenges in the form of hydrogen sulphide "eruptions" and Harmful Algal Blooms (HABs). Mariculture activities have intensified in Namibian waters in the last five years, during which time these naturally-occurring events have proven to be major hazards that the mariculture industry must contend with. Intensely low-oxygen conditions throughout the inshore water column coupled with hydrogen sulphide are not only characteristic of the sulphide events but also occur as a consequence of non-toxic high- density dinoflagellate blooms that die and decay along the coast. Although such conditions are sporadic and short- lived, a single event lasting longer than five days can have devastating impacts on the inshore sea life, including farmed shellfish. Such impacts in 2008 cost some oyster producers 80% of their annual production. The oceanographic conditions associated with these events suggest indicator or "early warnings" that could be used.HAB toxicity appeared to be coupled to the increased proportion of dinoflagellates in the total microalgae biomass in 2008, with unprecedented Diarrhetic Shellfish Poisoning (DSP) -positives in farmed shellfish (Currie, 2009).

As pressure on our resources grows and consumers become more aware of their environmental impact, the demand for sustainable products is growing. The demand for sustainable seafood in particular has grown exponentially, resulting in consumers looking to aquaculture products as a more sustainable alternative to wild caught products. Although often seen as a solution to over-fishing, aquaculture does have its

own suite of environmental problems which could challenge this perception and threaten the development of the industry. It is therefore important that aquaculture is done in a sustainable manner (Okes and Petersen, 2009).

The near collapse of the oyster industry in Walvis Bay can be hindered from happening again if an inland mariculture which involves producing oysters as well as algae will be put in practice. Therefore, the study was carried out to help pacific oyster farmers to decide on what diets to use when rearing oysters from spawning to spat and hence market size. The study concentrated on feeding oysters with different diets (*Pavlova lutherii,* and *Isochrysis* (T-ISO), the control was not fed) and in water temperature of 28 degree Celsius from spawning to spat. Measurements were taken on a daily basis.

2. PROBLEM STATEMENT

Oysters are slow growing animals and as far as commercial mariculture is concerned factors contributing to slow grow may be as a result of feed and/ or temperature. A bivalve hatchery is a business and like any other business it must be run efficiently and it must be economically viable. Government subsidies or grants may help offset costs particularly during initial stages of operation, but eventually the hatchery must stand on its own and be profitable.

The cost of such activities can be in a long run be escaped especially if the hatchery starts producing its own feed rather than pumping from the sea which might cost more than what the hatchery will produce. Producing own feed and starting up a land based mariculture venture will also minimize mortalities especially in the wake of algal blooms and or sulphur eruptions. It will always be safe if the diets are controlled as well the environmental factors such as temperature, salinity and pH.

3. RESEARCH OBJECTIVE

3.1 Research objective

- To determine and compare the growth rate of pacific oysters fed three different diets at 28°C.
- To measure growth, growth rate and specific growth rate

3.2 HYPOTHESIS

Hypothesis specification

H_{01}: there are no significant differences in mean shell length and growth
H_{11}: there are significant differences in mean shell length and growth

Level of significance: 0.05

4. Literature Review

In hatcheries, it is common practice to feed brood stock with live microalgae (either mono-specific or mixture diets) to achieve optimal food conditions. Microalgae culture represents one of the most delicate steps of the entire production cycle and requires considerable human and economic investment (Ponis *et al*. 2003).Commercial preserved concentrates of microalgae are sometimes used when live microalgae are not available. However, no studies have been made on conditioning Japanese oyster brood stock with either different temperature regimes or using preserved concentrates of microalgae in a closed re-circulating system.

Temperature and quality of the available food are important factors that influence the physiology of oysters; however, the combined effects have not been well studied. We evaluated the impacts of the temperature and diet on the growth, survival and biochemical composition in the Pacific oyster *Crassostrea gigas* spat, cultured in the laboratory for 8 weeks at 23, 26, 29 and 32°C and fed *Isochrysis* sp.-*Pavlova lutheri* and *Dunaliella tertiolecta*. The growth and biochemical composition showed a pattern, which changed in response to rising temperature. High temperatures achieved in the environment, as those reached on clear summer days during low tides, are an important stressor in oyster spat, especially when the quality of the available food is poor (Flores- Vergara *et al*,2004)).

The physiological components regulating intraspecific growth differences among individuals living in the same environment may be affected by differences in energy acquisition (food consumption and assimilation), differences in the allocation of energy among maintenance, growth, reproduction and other consuming activities, and differences in the metabolic cost of growth (Bayne, 1999). The energy budget or ‘scope for growth’ provides a means of integrating the basic physiological processes into an index of energy available for growth and reproduction. In bivalves, the scope for growth has proved to be an accurate predictor of total production, which includes growth rate and gamete production (Pernet *et al*, 2008).

"Temperature and concentration of particulate food are two of the main factors that affect bivalve filter feeders in relation to their growth, survival, gonad conditioning and physiology. Studies carried out on the physiology of bivalve filter feeders in response to broad ranges of microalgal concentrations and/or water temperature have shown that the filtration rates, ingestion, absorption, oxygen consumption and/or excretion become modified with changes in these parameters, and allow maintenance of high values in scope for growth within given ranges of the parameters depending on the species observed" (Velasco, 2006).

5. RESEARCH AND MATERIALS METHODS

5.1 STUDY AREA

The study was done at Beira aquaculture based at Mile 4, in Swakopmund, Namibia. The experimental units included newly spawned pacific oyster larvae. Three diets (*pavlova lutherii (pl), T-isocrysis (Tiso/ T-Iso) and No feed (NF))* were fed. The experiment was replicated four times.

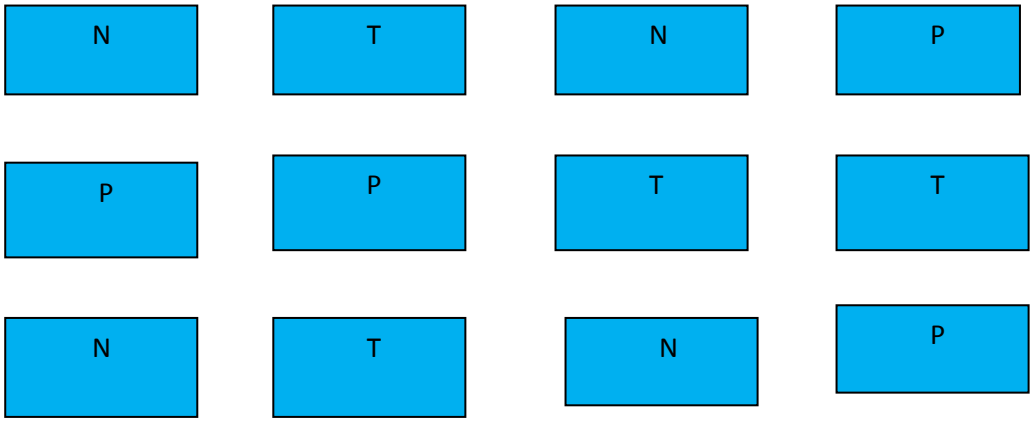
New larvae was used which was a result of spawning. Gamete stripping spawning technique was used where the brood stock was opened and gonads stripped into separate 1L containers. This was diluted and the suspensions (eggs and sperms) mixed when most of the eggs were round (a microscope was used to observe the egg shape).

The larvae were fed after 24 hours prior to spawning, and the treatment was randomly allocated to experimental units. A random- hat method was used.

Table 1: Treatment table

	Treatment x 4 replicates
Pavlova lutherii	Treatment 1 x 4
T-isochrysis	Treatment 2 x 4
NO FEED	Treatment 3x 4

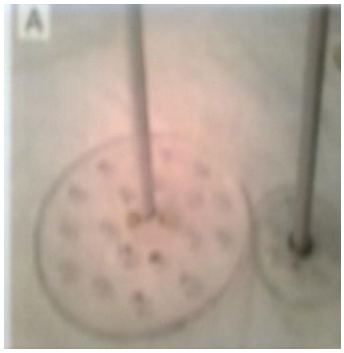
Arrangement of tanks

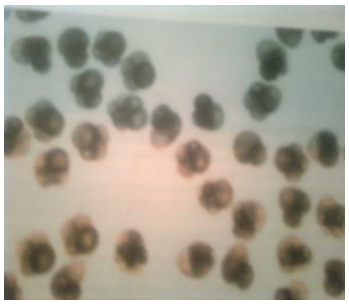


N No Feed

P *Pavlova*

T *Isochrysis*

(a) 

(b) 

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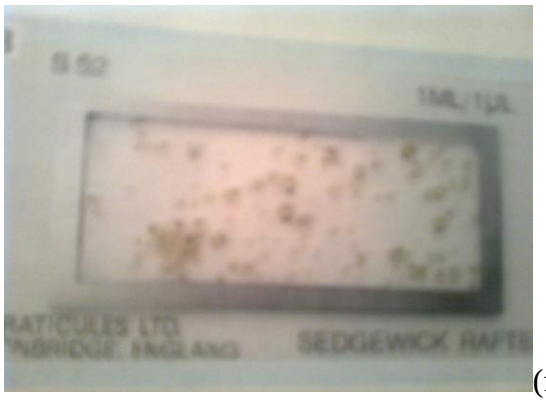
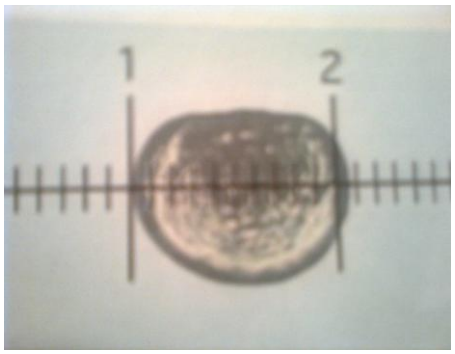
(e) 

(f) 

(g) 

(a) Plastic plunger used to stir the gamete mixture

(b) Division as seen under the microscope- the culture is then put into experimental tanks

(c) Experimental tanks where larvae was reared

(d) Sieve used to wash the larvae drained from the tanks

(e) Sedge wick rafter used to count the number of cells in a millimeter

(f) The lines across the larvae are the micrometer that was used to measure the shell length. The larvae are approximately 105 micrometer.

(g) The metamorphosis tank where the larvae that undergoes metamorphosis are put to help them pass through the metamorphosis stage fast by application of chemicals such as *epinephrine*.

The larval culture water was changed every morning and the larvae fed with 16 000 cells per millimeter of diet afterwards. Growth was measured in terms of shell length, and was taken daily for a duration of 30 days. Measurements were stopped after the larvae fed with *T-isochrysis* underwent metamorphosis to change into spats (benthic animals).

Growth rate was measured using the microscope fitted with a micrometer. 1L of larvae was taken from each tank at random of which the mean was calculated and analyzed at the end of the experiment.

The experiment was done in a static system. The tanks were near the air source. Aeration is very important because it is the source of the oxygen that is needed by larvae to respire and for the distribution of food within the water column. The water temperature was measured using a thermometer.

5.2 Data collection

Data was collected daily (in the mornings). Data was recorded for 40 days, but only 30 days data was used in the analysis due to the fact that larvae fed with *Tiso* underwent metamorphosis on the 30^{th} day thus letting it be out of the study. Since this study is a comparison that concerns three diets, one diet cannot be thrown out of the study as all have to be compared as soon as one undergoes metamorphosis. Hence the raw data collected for 30 days was the one that was used for analysis.

5.2.1 SAMPLING PROCEDURES

A sample of water containing oyster culture was taken using a plastic pipette. These data was recorded on the collection form. A diet given to the larvae in different tanks was also recorded. One millimeter of culture water was taken from each tank using an epperndorf automatic pipette and the larvae in each sample were accounted for shell length measurement using a Sedgwick rafter and micrometer fitted microscope. These data was recorded on the collection form. Temperature and diet type given to the larvae in different tanks was also recorded.

5.2.2 Growth and mortality indices

Growth (micrometers) = $L_t - L_i$

Growth rate (micrometer/day) = $(L_1 - L_t)/T$

Biomass yield (total length increment) = $S*(L_t - L_i)$

Where:

Li= initial shell length (micrometers)

Lt= final shell length (micrometers)

T= number of days

S= number of survivors per diet

Amount of feed consumed per day= initial no. of cells/mL- final no. of cells/mL

Mortality (number of dead larvae) = to be determined in a mL using a microscope

5.2.3 LABORATORY AND STATISTICAL ANALYSIS

The samples were measured (that is, the length of the shell recorded) within an hour at random, starting with any tank. The samples were put on a slide or Petri dish and observed under the microscope, the length was measured using the microscope fitted with a micrometer.

Quantitative data was collected and since it is a one - factor experiment in a Completely Randomized Design (CRD) to be replicated four times, it was analyzed using analysis of variance (ANOVA) to compare the growth rate of larvae at the end of the experiment. A P-value <0.5 was considered significant.

6. RESULTS AND DISCUSSION

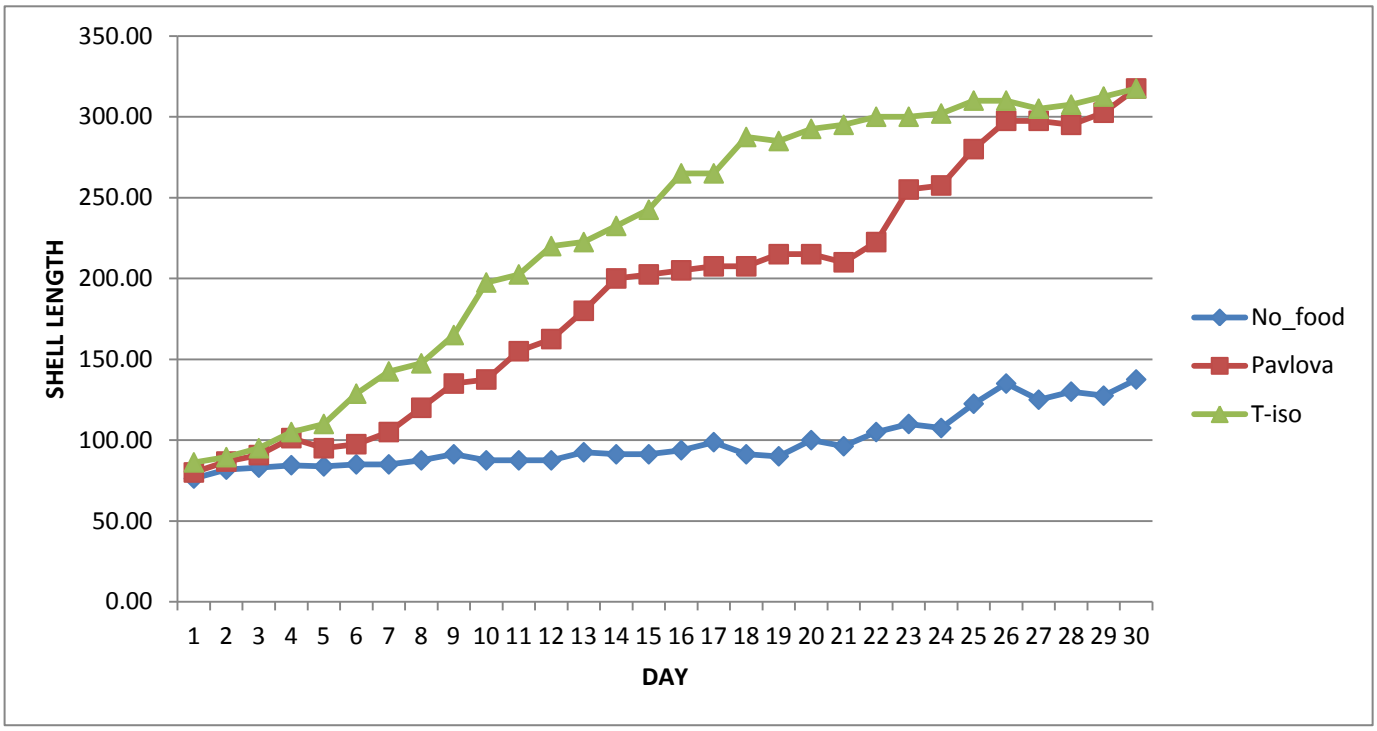


Figure 1: Graph depicting the growth of pacific oyster larvae given different diets over a period of 30 days

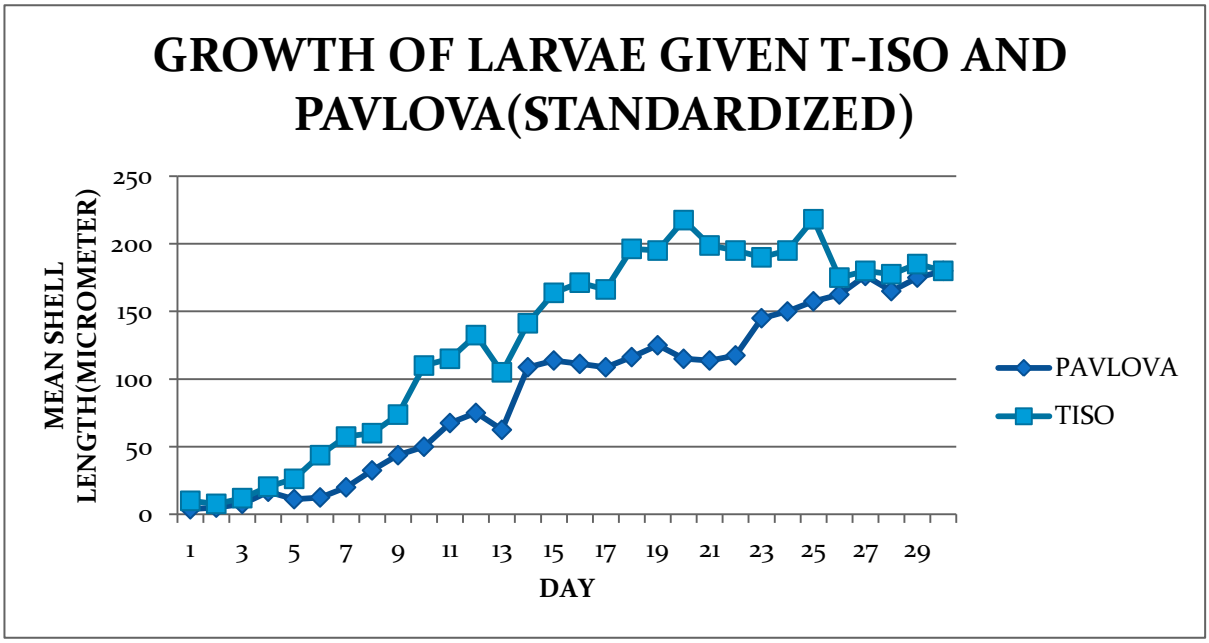


Figure 2: Standardized data for the growth of pacific oyster larvae given different diets over a period 30 days

As stated above the study was done for forty days but only data collected in thirty days was used for analysis due to the metamorphosis stage that was reached on the thirtieth day of the study by larvae that was fed with *T-iso.*

The stocking density decreases after the thirtieth day study period by thirty percent, twenty percent and ten percent for *Pavlova, T-isochrysis* and No feed respectively.

There was a significant difference ($P<0.05$) in growth (shell length increment) of *Crassostrea gigas* fed *PL, Tiso* and no feed was observed. However, oyster larvae fed with *Tiso* showed better growth performance, in terms of shell length commercially, especially from day five until day twenty nine specifically between *PL* and *Tiso* (Figure 1). Difference in shell increment with regard to No feed can be observed from the very beginning of the experiment day. Higher growth rate was obtained for *C.gigas* fed with *Tiso* than that fed with *PL* and the ones that were not fed. Tiso and pl had the same mean shell length on the 30^{th} day, but *Tiso* underwent metamorphosis while *Pl* did not at that stage.

Results from the graph drawn from the raw data (figure 1) showed that at the beginning of the feeding trial there was no significant difference in the shell length increment of the larvae. However, after about five days there was a significant difference observed amongst the larvae of a different diet. *Tiso* recorded the highest growth gained (shell length increment) (277.5 micrometers, plus reaching metamorphosis stage first) and *no feed* recorded the lowest growth gained (shell length increment) (97.5 micrometers) (Table 2).

Results from ANOVA calculations (appendix) also showed that there is a significant difference ($P<0.005$) between the different diets and that *Tiso* induced greater growth followed by *PL* then no feed.

A high survival rate was recorded for the larvae that were not fed. The water had nutrients that induced the growth allowing the larvae to survive (Table 2; figure 3 and figure 4) for more than 30 days. The diets had an equal chance of being fed on since oyster larvae are filter feeders. The nutritional composition of *Pl* has a high percentage of protein, but induced less growth and slow metamorphosis stage. This may be that the larvae synthesized the protein in *Tiso* more easily or faster than that in *Pl* (table 3).

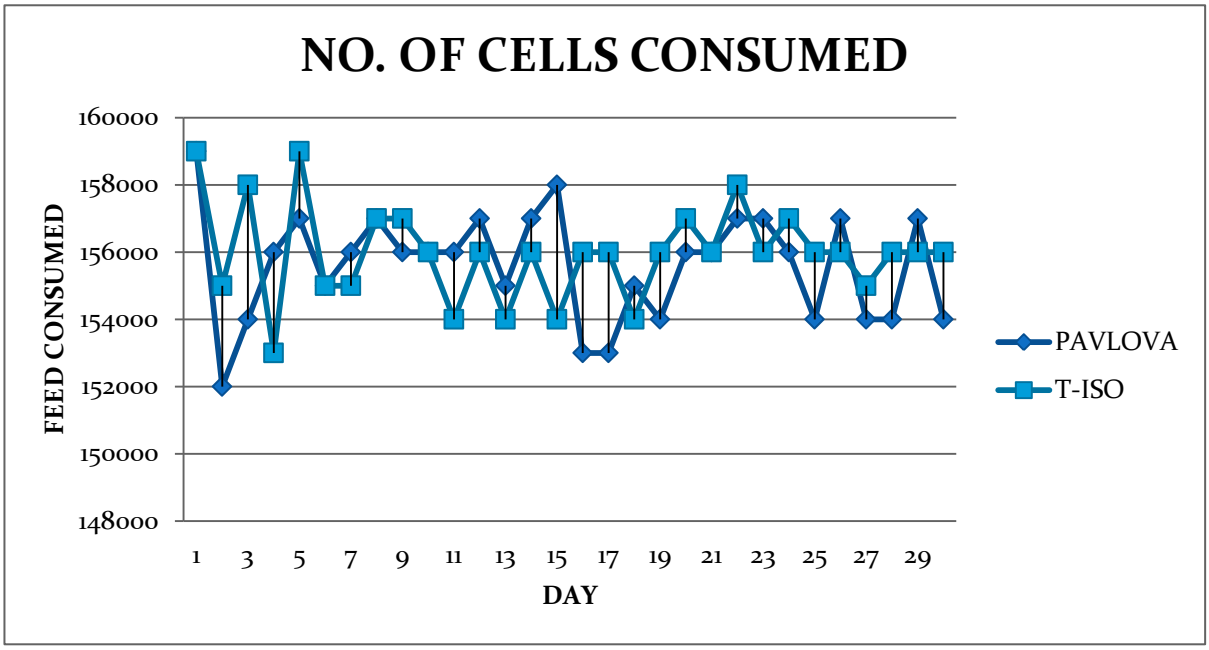


Figure 3: Feed consumed by the experimental larvae over a period of 30 days

Table2. The growth indices of the experimental oyster larvae obtained in the investigation are presented in table 2 below: Stocking density, survival, shell length increment (growth), growth rate and biomass yield for larvae (*C. gigas*) cultured at three diets over thirty days.

		TREATMENT		
		PAVLOVA	***T-ISO***	***NO FEED***
STOKING DENSITY	INITIAL	40	40	40
	FINAL	28	32	36
SURVIVAL (%)		70	80	90
Initial shell length after spawning (micrometer)		20	20	20
SHELL LENGTH(SL) In micrometer	INITIAL(Before being fed)	40	40	40
	FINAL(day 30)	317.5	317.5	137.5
GROWTH		277.5	277.5	97.5

GROWTH RATE(in micrometer)		9.25	9.25	3.25
BIOMASS YIELD(Micrometer)		194.25	222	87.75

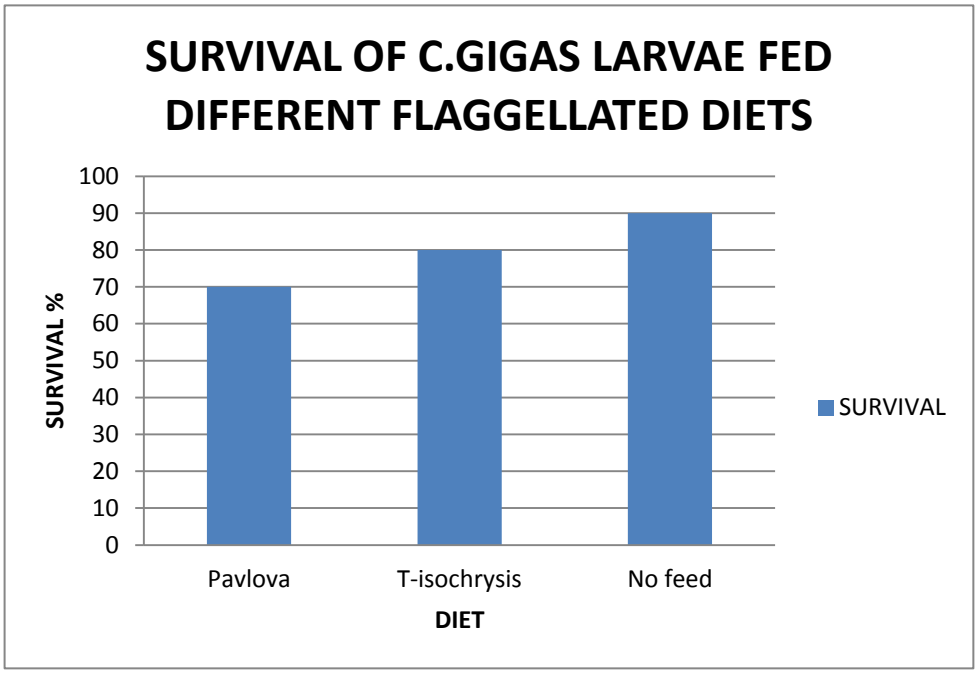


Figure 4: Survival of *C.gigas* fed different diets for thirty days in a closed system (tanks)

Table 3: Nutrient composition

MACRONUTRIENTS (%)	**PAVLOVA**	**T-ISO**
	COMPOSITION	
Chlorophyll a	0.84%	0.97%
Protein	29 03%	22.89%
Carbohydrate	8.89%	6.06%
Fat	12.02%	19.86%

Source: FAO

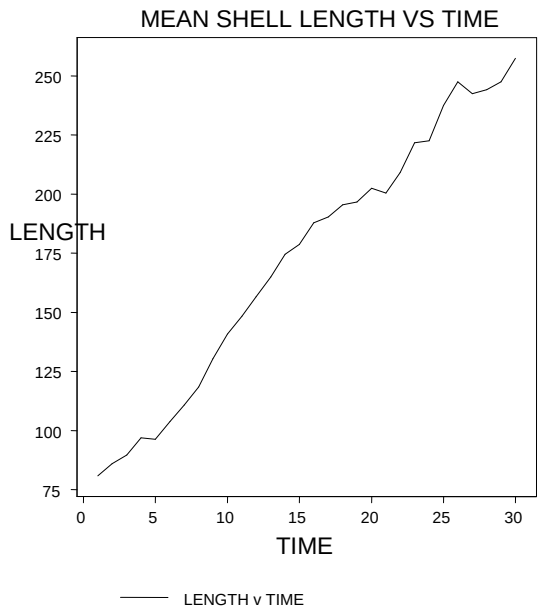


Figure 5: Graph depicting growth with time

Limited data exist for the growth parameter of *C. gigas* larvae especially in Namibia. Results of this study revealed that oyster larvae (*Crassostrea gigas*) fed *Tiso* had a higher growth than *PL* and *NO FEED. Tiso* induced a faster metamorphosis which took place on day thirty. After the thirtieth day the larvae that underwent metamorphosis was taken out of experimental tanks thus out of the study. *Pl* underwent metamorphosis outside the study period on the thirty-eighth (38^{th} day). By day forty (40), the larva that was not fed did not still undergo metamorphosis and its average shell length was two hundred and ten (210). Feed consumption in larvae fed *T-Iso* and *Pavlova* was the same. This indicates that both feed types were equally attractive and palatable BUT better growth and an earlier metamorphosis stage in oysters fed *T-Iso* indicates that *T-Iso* was nutritionally more complete. Might also be due to a higher energy supply in *T-Iso* but this needs further research to be confirmed.

Tiso therefore showed that it is a more suitable flagellate to be used in hatcheries that rear oyster larvae in comparison to *Pavlova*. The use of both flagellates at the beginning of larvae rearing is wasteful and requires a lot of space as well as labour and operation costs.

7. MANAGEMENT RECOMMENDATION

Larval rearing companies should undergo one flagellate which is *pavlova lutherii* to use *t-isochrysis* instead of using both to reduce costs and space as well as labour costs. Companies like Beira Aquaculture should decrease costs by not using a combination of T-iso and Pavlova (they are all flagellates and are of the same size) and rather focus on T-iso only.

Start producing own feed rather than pumping from the sea which might cost more than what the hatchery will produce

Further studies are recommended to incorporate temperature and any other environmental parameter such as salinity and further include the shell length after metamorphosis as well as the other parameters that contribute to or affect the growth of oysters.

8. CONCLUSION

- The study has showed that *C.gigas* oyster larvae fed with *Tiso* had a faster growth than that fed with *Pavlova* with respect to the control diet, *No feed*. Survival was greater for *C. gigas* that was not fed though. The results may have been as they are, depending on the digestibility of *Tiso* compared to *Pavlova* and *No feed*. The feed had an equal chance of being eaten or fed upon as the feed consumed data indicate (appendix). Recommendation: Production of own feed seems promising. Limits effects of inhibitory pumping costs, electricity, algal blooms, sulphur eruptions, and temperature fluctuations.

9. ACKNOWLEGDEMENTS

Thanks to the Almighty God for the love, peace and strength given to me during the study and beyond. I would like to acknowledge the following people for their tremendous support and contributions towards the success of this research project. The financial support of the Department of Fisheries and Aquatic sciences and its project coordinator Mr M. Tjipute is gratefully acknowledged. I would like to specially thank Mr J. A. Esterhuizen and Mr. R. M. Romero for supervising and providing assistance in running the experiment. Mr. R. M. Romero did the recording in my absence as well as changing the larval culture water (cleaning) and feeding them, not to mention allowing this study to be done at his aquaculture company namely Beira Aquaculture. A lot of thanks to the Head of Department of Fisheries and Aquatic science, Mr. L. Kandjengo for allowing this study to be carried out; Department of Fisheries and Aquatic Sciences Staff, and classmates.

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11. APPENDIX

a) Feed consumed by the experimental units

DAY	Diet	FEED CONSUMED
1	Pavlova	159000
1	T-iso	159000
1	No food	0
2	Pavlova	152000
2	T-iso	155000
2	No food	0

3	Pavlova	154000
3	T-iso	158000
3	No food	0
4	Pavlova	156000
4	T-iso	153000
4	No food	0
5	Pavlova	157000
5	T-iso	159000
5	No food	0
6	Pavlova	155000
6	T-iso	155000
6	No food	0
7	Pavlova	156000
7	T-iso	155000
7	No food	0
8	Pavlova	157000
8	T-iso	157000
8	No food	0
9	Pavlova	156000
9	T-iso	157000
9	No food	0
10	Pavlova	156000
10	T-iso	156000
10	No food	0
11	Pavlova	156000
11	T-iso	154000
11	No food	0
12	Pavlova	157000
12	T-iso	156000
12	No food	0
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14	T-iso	156000
14	No food	0
15	Pavlova	158000
15	T-iso	154000
15	No food	0
16	Pavlova	153000
16	T-iso	156000
16	No food	0
17	Pavlova	153000
17	T-iso	156000

17	No food	0
18	Pavlova	155000
18	T-iso	154000
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19	Pavlova	154000
19	T-iso	156000
19	No food	0
20	Pavlova	156000
20	T-iso	157000
20	No food	0
21	Pavlova	156000
21	T-iso	156000
21	No food	0
22	Pavlova	157000
22	T-iso	158000
22	No food	0
23	Pavlova	157000
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30	Pavlova	157000
31	No food	0
31	Pavlova	155000
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35	Pavlova	156000
36	No food	0
36	Pavlova	157000
37	No food	0
37	Pavlova	1554000
38	No food	0
39	No food	0
40	No food	0

(b) MEAN SHELL LENGTH

DAY	No_food	*Pavlova lutherii*	*T-isochrysis*
1	76.25	80.00	86.25
2	81.75	86.75	89.50
3	83.00	90.75	95.00
4	84.50	101.25	105.00
5	83.75	95.00	110.00
6	85.00	97.50	128.75
7	85.00	105.00	142.50
8	87.50	120.00	147.50
9	91.25	135.00	165.00
10	87.50	137.50	197.50
11	87.50	155.00	202.50
12	87.50	162.50	220.00
13	92.50	180.00	222.50
14	91.25	200.00	232.50
15	91.25	202.50	242.50
16	93.75	205.00	265.00
17	98.75	207.50	265.00
18	91.25	207.50	287.50
19	90.00	215.00	285.00
20	100.00	215.00	292.50
21	96.25	210.00	295.00
22	105.00	222.50	300.00
23	110.00	255.00	300.00
24	107.50	257.50	302.00

25	122.50	280.00	310.00
26	135.00	297.50	310.00
27	125.00	297.50	305.00
28	130.00	295.00	307.50
29	127.50	302.50	312.50
30	137.50	317.50	317.50

(c) Shell length increment

	SHELL LENGTH INCREMENT: INITIAL SHELL LENGTH(40)- LENGTH AT FIRST METAMORPHOSIS		
	INITIAL length	FINAL length	LENGTH increment
DIET			
PAVLOVA	40	317.5(day 30)	277.5
TISO	40	325(day 39)	285
NO Food	40	175(day 40,no metamorphosis)	135

(d)Mean shell length vs time

TIME	LENGTH
1	80.83
2	86.00
3	89.58
4	96.92
5	96.25
6	103.75
7	110.83
8	118.33
9	130.42
10	140.83
11	148.33
12	156.67
13	165.00
14	174.58
15	178.75
16	187.92
17	190.42
18	195.42
19	196.67
20	202.50
21	200.42
22	209.17

23	221.67
24	222.50
25	237.50
26	247.50
27	242.50
28	244.17
29	247.50
30	257.50

22
(f) ANOVA RESULTS
Analysis of variance (ANOVA)
Source of variation d.f. s.s. m.s. v.r. F pr.
Subject stratum
Diet 2 1063052.4 531526.2 1166.73 <.001
Residual 9 4100.1 455.6 1.77
Subject.Time stratum
d.f. correction factor 0.1588
Time 29 1101633.5 37987.4 147.81 <.001
Time.Diet 58 342637.1 5907.5 22.99 <.001
Residual 261 67077.1 257.0
Total 359 2578500.3
(d.f. are multiplied by the correction factors before calculating F probabilitie
s)
***** Tables of means *****
Grand mean 172.68
Time 1 2 3 4 5 6 7
80.83 86.00 89.58 96.92 96.25 103.75 110.83
Time 8 9 10 11 12 13 14
118.33 130.42 140.83 148.33 156.67 165.00 174.58
Time 15 16 17 18 19 20 21
178.75 187.92 190.42 195.42 196.67 202.50 200.42
Time 22 23 24 25 26 27 28
209.17 221.67 222.50 237.50 247.50 242.50 244.17
Time 29 30
247.50 257.50
Diet No food Pavlova T-iso
98.85 191.12 228.07
Time Diet No food Pavlova T-iso
file:///D|/Project%20Report.%20Sophia%20M%20Isala/5.ANOVA%20results.txt[3/11/2013 12:12:30 PM]
1 76.25 80.00 86.25
2 81.75 86.75 89.50
3 83.00 90.75 95.00
4 84.50 101.25 105.00
5 83.75 95.00 110.00
6 85.00 97.50 128.75
7 85.00 105.00 142.50
8 87.50 120.00 147.50
9 91.25 135.00 165.00
10 87.50 137.50 197.50
11 87.50 155.00 202.50
12 87.50 162.50 220.00
13 92.50 180.00 222.50
14 91.25 200.00 232.50
15 91.25 202.50 242.50
16 93.75 205.00 265.00
17 98.75 207.50 265.00
18 91.25 207.50 287.50
19 90.00 215.00 285.00

20 100.00 215.00 292.50
21 96.25 210.00 295.00
22 105.00 222.50 300.00
23 110.00 255.00 300.00
24 107.50 257.50 302.50
25 122.50 280.00 310.00
26 135.00 297.50 310.00
27 125.00 297.50 305.00
28 130.00 295.00 307.50
29 127.50 302.50 312.50
30 137.50 317.50 317.50
*** Standard errors of means ***
Table Time Diet Time
Diet
rep. 12 120 4
e.s.e. 4.628 1.948 8.118
d.f. 41.43 9 45.87
Except when comparing means with the same level(s) of
Diet 8.016
d.f. 41.43
Correction factors have been applied to residual d.f.
(see analysis-of-variance table for details)
*** Standard errors of differences of means ***
Table Time Diet Time
Diet
rep. 12 120 4
s.e.d. 6.545 2.756 11.481
d.f. 41.43 9 45.87
Except when comparing means with the same level(s) of

Diet 11.336
d.f. 41.43
Correction factors have been applied to residual d.f.
(see analysis-of-variance table for details)
*** Least significant differences of means (5% level) ***
Table Time Diet Time
Diet
rep. 12 120 4
l.s.d. 13.213 6.233 23.112
d.f. 41.43 9 45.87
Except when comparing means with the same level(s) of
Diet 22.886
d.f. 41.43
Correction factors have been applied to residual d.f.
(see analysis-of-variance table for details)
***** Stratum standard errors and coefficients of variation *****
Stratum d.f. s.e. cv%
Subject 9 3.897 2.3
Subject.Time 261 16.031 9.3