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Pond production of *Artemia* in a solar salt work in Kenya

By

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LIST OF ACRONYMS

*	Asterisk
>	Greater than
=	Equal to
±	Plus or minus
%:	Percent
∑:	summation
µg/L:	Microgram per liter of water
µm:	Micrometer
am:	Ante meridiem
ANOVA:	Analysis of variance
BOD:	Biological oxygen demand
C:	Carbon
cDNA:	Complementary DNA - is a single-stranded DNA that is complementary to a certain sequence of messenger RNA
cm:	Centimetres
DAP:	Diammonium phosphate
DPA	Docosapentaenoic acid
DW:	Dry weight
EDTA:	Ethylenediaminetetra-acetic acid ($[(HOOCCH_2)_2NCH_2]_2$)
etc:	And so on
g:	Gram
GSL:	Great Salt Lake
GW:	Green Water
GW+PM:	Green water supplemented with pig manure
GW+SBM:	Green water supplemented with soybean
ha:	Hectare
h:	Hour
H_2O:	Water
kg:	Kilogram
km:	Kilometer
KMFRI:	Kenya Marine and Fisheries Research Institute

Kn1:	Kenya *Artemia* strains collected in 2010
Kn2:	Kenya *Artemia* strains collected in 1996
L:	Litre
m:	Meter
mg:	Milligram
mL:	Millilitre
mm:	Millimeter
N/P:	Nitrogen to phosphorus ratio
N:	Nitrogen
$^{\circ}$C:	Degrees centigrades
m^2:	Meter square
m^{-2}:	Per meter square
m^3:	Cubic meter
m^{-3}:	Per cubic meter
P:	Phosphorous
p:	P-value
pH:	Power of hydrogen
Ppt:	parts per thousands
&:	And
PM:	Pig manure
pm:	Post meridiem
RB:	Rice bran
SFB:	San Francisco Bay *Artemia* strain
sp:	Species
StDev:	Standard deviation
SW:	Sea water
VC:	Vinh Chau strain
Day 0:	Inoculation day
USA:	United States of America
WW:	Wet weight

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study was conducted in a solar salt work in Malindi, Kenya with the aim to investigate the effect of feeding *Artemia franciscana* (Vietnam strain) with fertilized and un-fertilized green water on its growth and reproduction through parallel production runs over three months. Additionally, a laboratory culture test aimed to study the effect of temperature (28, 32 and 36°C) on survival, growth and reproduction and the thermotolerance of *A. franciscana* presently occurring in the saltworks in Kenya, in comparison with San Francisco Bay (SFB) and Vinh Chau (VC) *Artemia*. The objective was to observe to what extent the Malindi *A. franciscana* has adapted to the local conditions over the 2 decades since it has been inoculated. Questionnaires were submitted to the local population and aimed at assessing the valuability of the project to the community and level of awareness of *Artemia* culture practices in Malindi.

In the field, significantly high ($p<0.05$) differences in nutrient parameters in the fertilized culture ponds and fertilized reservoir ponds especially during the third month were recorded. Significantly ($p<0.05$) higher growth, population density, earlier maturation and fecundity were observed in the fertilized culture ponds as compared to the control ponds. This indicates that fertilization of the *Artemia* ponds is of utmost important for better production and maximizing reproductive parameters. Adding supplemental feeds in the culture ponds further helps in sustaining *Artemia* production.

For the laboratory tests, one-way ANOVA revealed a significant ($p<0.05$) interaction between strain and temperature, for most of the reproductive parameters. SFB strain seemed to survive better at 28°C compared to other strains while Kenyan strains performed even better than VC at 32°C. None of the strains performed well at 36°C. Due to this better response of the Kenyan strains (especially Kn2) and the VC strain they may be regarded as the most superb strains for production in the salt works in the Malindi area. Finally, the questionnaire results showed that 95% of the villagers were aware of the *Artemia* culture initiatives and three quarters of the interviewees showed their appreciation to the *Artemia* culture project, which gives good hopes that the *Artemia* production techniques will on the long run be implemented by the local rural population.

1 INTRODUCTION

Brine shrimp *Artemia* has been portrayed by several scientists (Persoone and Sorgeloos, 1980; Lenz, 1987) as a crustacean that has established itself worldwide in a harsh saline aquatic environment. Also, *Artemia* has been defined as a non-selective filter feeder, filtering microalgae, detritus and bacteria, limited by the size of the ingested particles (Van Stappen, 1996; Fernández, 2001). *Artemia* has been familiar to man for centuries, even though the use of *Artemia* in larviculture of fish and shrimps started only in the 1930's when scientists found that it is a superb food for newly hatched fish larvae (Bengtson et al., 1991) and ornamental fish (Lim et al., 2001; 2003). In addition, *Artemia* biomass is regarded as excellent nursery food for marine fish, shrimp, prawn and crab (Merchie, 1996; Sorgeloos et al., 1998; Dhont and Sorgeloos, 2002).

Generally, *Artemia* is a high protein ingredient for aquaculture feeds, and activates maturation in shrimp broodstock (Naessens et al., 1997; Wouters et al., 2002). Thanks to its highly nutritional value, its convenience coupled with fast aquaculture development, its use has fastly spread worldwide (Bengtson et al., 1991).

According to Sorgeloos et al. (1991), live foods are a vital food source for the larvae of cultured species particularly those without fully developed digestive system; live food organisms, and especially *Artemia,* offer digestive enzymes that breakdown the food ingested by larvae. *Artemia* meets both the dimensional (in terms of prey size) and nutritional requirements of the fish or finfish larva (Lubzens et al., 1989; Sorgeloos et al., 1991). Moreover, the nutritional value of *Artemia* can be improved by the enrichment process, thereby incorporating fatty acids and other compounds into the larval rearing protocols (Sorgeloos et al., 1991).

Artemia can be produced in solar saltwork ponds to retain a 'healthy' biological salt production process. *Artemia* in salterns controls the algal blooms, hence preventing contamination of the salt. Moreover, *Artemia* plays a big role in solar salt production through releasing its metabolites which act as nutrient for the development of the red *Halobacterium* within the crystallization ponds, which increases heat absorption hence increasing the amount of salt production. Therefore, good management of the *Artemia* population in solar salterns shows the way to optimal salt production quality and quantity and moreover to the production of valuable by-products, counting cysts and biomass. *Artemia* production has been an economic success story in several places especially in the Vinh Chau area, Mekong Delta, Vietnam, where integrated production of *Artemia* in saltworks, combined with aquaculture, has become a thriving economic activity, and a wealth of scientific knowledge and practical experience has been gained.

Artemia was first introduced in Kenya in the period 1984-1986 through a collaborative project with the Kenya Marine and Fisheries Research Institute (KMFRI), funded by the Belgian Agency for Development Cooperation (BADC), aiming to study the potential of *Artemia* production in saltworks in the Malindi area. Although the technical feasibility of local *Artemia* production was demonstrated by the project, the execution of the application of *Artemia* in local larviculture as planned to be completed in the second phase was not done and ever since scientific research was not performed. The first inoculation was done with *A. franciscana* (there was no native strain), which established itself since. *Artemia* introduction in Kenya and Vietnam was done at about the same time but whereas Vietnam is now one of the top producers of pond produced

Artemia cysts in the World (Anh, 2009, Anh et al., 2009, Anh et al., 2010), there has been stagnation in aquaculture development in Africa (Bossier et al., 2010).

This Masters dissertation work fits within the framework of the *Artemia* project named ***"Improvement of the living standard of rural communities in Kenya through Artemia production in coastal saltworks"*** that is recently running at Gongoni-Malindi in cooperation with Kenya Marine and Fisheries Research Institute (KMFRI) and Ghent University, Faculty of Bioscience Engineering, Department of Animal Production, Laboratory of Aquaculture & *Artemia* Reference Center and which is financed as VLIR OI (Flemish Interuniversitary Council Own Initiative) project (Bossier et al., 2010).

The project is aimed at improving the Kenyan rural communities' living conditions through the pond production of *Artemia* cysts and biomass and by application of *Artemia* cysts and biomass in fast rising larviculture initiatives. The pros of integrating the production of salt and *Artemia* are expected to be verified in a pilot unit. Locally produced *Artemia* cysts and biomass are of utmost importance for the optimal local development of shrimp and fish larviculture. Local community development centres, which have already developed to some extent in the past very extensive aquaculture initiatives for the benefit of rural communities, are expected to be the target of demonstration of project activities throughout the project lifetime.

The study of this thesis was divided into two main parts, a field component and a laboratory component. The field component was designed with the aim to investigate in the field pilot unit ponds the effect of feeding *Artemia* with fertilized and un-fertilized green water on its growth and reproduction. The laboratory component aimed to study the effect of temperature on growth, survival and reproduction of the Kenyan strains and the thermotolerance of *A. franciscana* from Kenya. The former was analyzed in a culture test at various temperatures, the later in a standard thermotolerance test. By including in both tests, San Francisco Bay from USA (SFB) and Vinh Chau from Vietnam (VC) *Artemia* as control we aimed to observe to what extent the Malindi *A. franciscana* has adapted to the local conditions over the 2 decades since it has been inoculated.

Additionally, a questionnaire was distributed with questions related to awareness and general appreciation of the project. The aims of the questionnaire were;

- To assess the valuability of the project to the community where the *Artemia* project is running, and the potential economic importance of the project in terms of food security, income and expenditure
- To estimate the levels of awareness on *Artemia* culture practices in Malindi, Kenya.

The specific objectives of the field and laboratory tests were;

- To compare the growth and reproduction of *Artemia* in the fertilized and non-fertilized ponds
- To observe phyto/zooplankton species found throughout the culture system (including culture ponds, the water supply creek and the reservoir ponds).
- To observe how Kenyan, *A. franciscana* strains have adapted to elevated temperatures in the Malindi area by evaluating the effect of temperature on survival life span and reproductive characteristics and of thermotolerance of the *Artemia franciscana* strains established in the Malindi saltworks as compared with SFB and VC *Artemia franciscana.*

2 LITERATURE REVIEW

2.1 A*rtemia* biology

2.1.1 Morphology

Due to the fact that *Artemia* is not difficult to culture, and having a short generation time, it has been broadly used as a most appropriate test organism for elementary research, ranging from molecular biology to ecology, developmental physiology, biology, toxicology and aquaculture research.

The following is the systematic classification of *Artemia* genus in Animalia kingdom, as accepted today: Phylum: Arthropoda, Class: Crustacea, Subclass: Branchiopoda, Order: Anostraca, Family: Artemiidae, Genus: *Artemia*, Leach 1819.

Sorgeloos (1980a, 1986) described the morphology of adult *Artemia* as having a lengthened body of between 8 to 10 mm, a pair of complex stalked eyes, antennae which are used as sense organ together with 11 pairs of thoracopods and a straight digestive tract. At the anterior end of the head, brine shrimp *Artemia* consists of five coalesced segments (Fox, 2006). The second antennae are larger and differ between sexes morphologically. In adult males they are very large and modified to form a clasping organ to hold the female during copulation while females' second antennae are smaller (Figure 2.1). Females possess a conical pouch called the brood sac or ovisac which may store eggs or nauplii, but males bear a pair of tubular, retractile penisees; just behind the last pair of phyllopods (Fox, 2006).

Artemia's head possesses two; egg-like, protruding mandibles (Figure 2.1) and segmented appendages (Fox, 2006). The maxillae are used as a tool to transfer food from the thoracic appendages to the mouth. The excretory structures in adults are the two maxillary glands, or coxal glands, located in the segment of the second maxillae. The rest of the body is the segmented trunk made up of an anterior; limb-bearing thorax and limbless posterior abdomen. The thorax is composed of 11 independent segments with no carapace and no cephalothorax. The phyllopods are appendages in which the exoskeleton is thin, flexible, and blood pressure is needed to make sure that the appendages are kept stiff. These phyllopods are responsible for swimming, feeding, and respiration. The abdomen is made up of six segments, telson (with anus), and caudal furca (Fox, 2006).

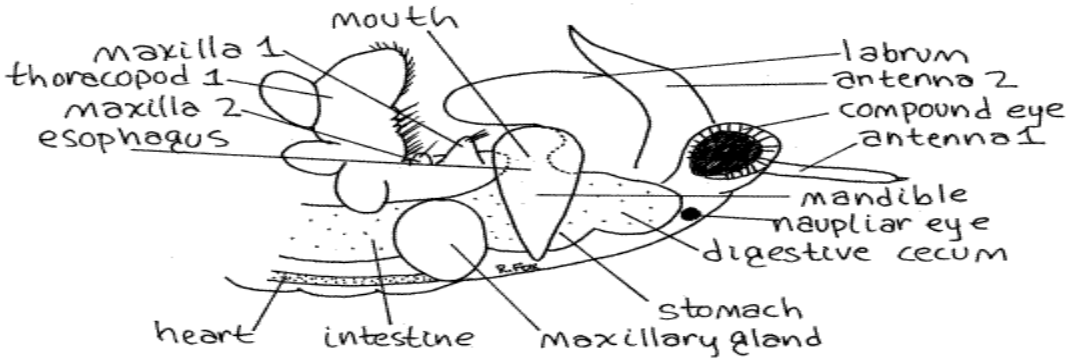


Figure 2. 1 Lateral view of the left side of the head and anterior thorax of a female *Artemia*, adopted from Fox (2006*)*.

2.1.2 *Artemia* life cycle

There are sexual and parthenogenetic *Artemia* populations. In bisexual populations, the common criteria used to differentiate the two sexes is the recognition of the presence of the hooked claspers, that can be observed from instar X in males, and the uterus in females that is situated between the anterior part and posterior part (abdomen) of the body (Sorgeloos et al., 1986), and where eggs are fertilized during copulation (Vos and de La Rosa, 1980).

Normally, free swimming nauplii (0.45 mm) (ovoviviparous reproduction) will be released from the brood sac of the female (Figure 2.2) after the eggs' development, or alternatively when attaining the gastrula stage, they are bounded by a multifaceted non-cellular shell (Morris and Afzelius, 1967; Anderson et al, 1970) and are set down as cysts (oviparous reproduction) (Clegg and Conte, 1980).

Generally, *Artemia* released from the embryo as cysts stays in a condition of diapause. After deactivation of the diapause state, the cyst enters into a state of quiescence where the metabolism can be resumed when the cyst is placed into advantageous conditions that can lead to hatching (Van Stappen, 1996).

In this case, the metabolic activities in the cell start again and after about 6-8 h the cyst's outer membrane bursts, the embryo becomes visible, surrounded by the hatching membrane and the eye of the instar I nauplius now becomes visible (Sorgeloos, 1980a). There are three pairs of appendages: antennae having a locomotory role, antennules as a sensory organ, and the simple mandibles (Sorgeloos, 1980a). Instar I stage of *Artemia* depends totally on its yolk reserve and its digestive system is not yet entirely developed at this time (Anh, 2000).

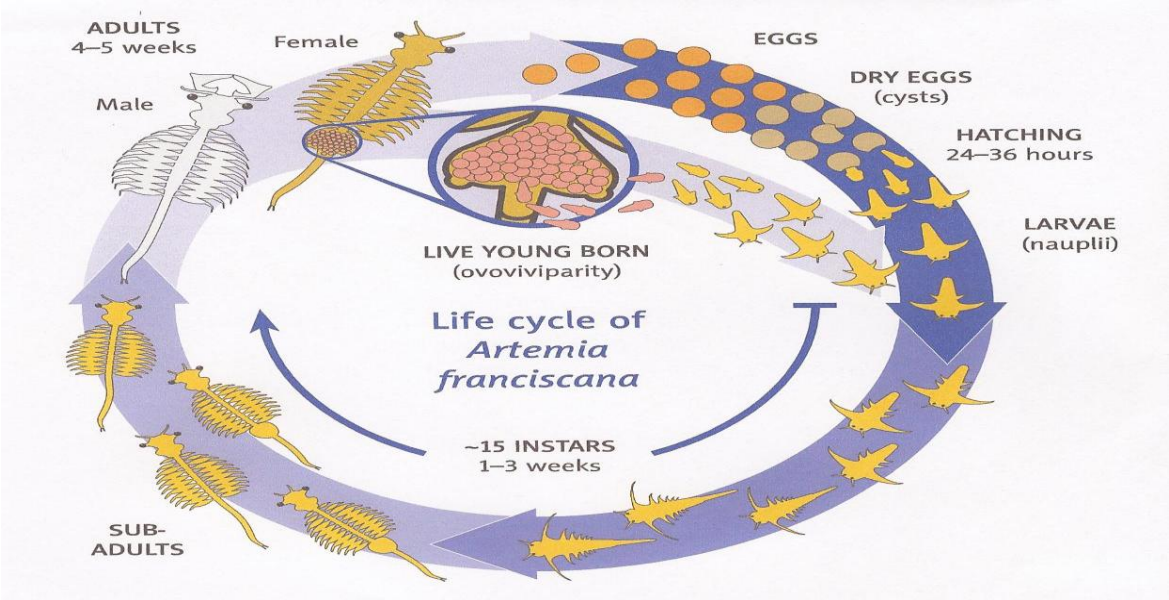


Figure 2. 2 Life cycle of *Artemia franciscana*, adopted from Madden (2009).

According to Anh (2000), it may take up to 8 h for the instar I to moult into the instar II nauplius and during this stage it is capable of filtering small particles such as bacteria, detritus and microalgae using its second antennae, as the yolk reserve is now depleted. In addition,

Schrehardt (1987) reported that the post-embryonic development takes up to 15 molts before the larvae reach the adult stage (Figure 2.2). Provided that the environmental factors are conducive, the development from the nauplius to the adult stage takes about 8 days. *Artemia* can survive for several months and a new batch of offspring may be produced by the female every 5 days, consisting of up to 200-300 individuals (Van Stappen, 1996; Vos and de La Rosa., 1980).

Reproduction in *Artemia* is either ovoviviparous or oviparous: embryos may develop inside the eggs that are retained within the mother's body until they are ready to hatch (ovoviviparous reproduction). In parthenogenetic populations, there is no need of fertilization and the embryo develops shortly after the eggs reach the uterus (Sorgeloos et al., 1986). In oviparous reproduction, soon after mating, it takes about 40 min to 1 h for the eggs to be fertilized before they are released into the water (Bowen, 1962) while in ovoviviparous reproduction, they are released into the water as nauplii (Figure 2.2) (Vos and de La Rosa., 1980). A female *Artemia* is capable of changing between oviparity and ovoviviparity in between batches (Clegg and Conte, 1980; Vos and de La Rosa, 1980; Criel, 1992; Anh, 2000).

D'Agostino (1965) and D'Agostino and Provasoli (1968) emphasized that food quality and/or amount of food tend to stimulate oviparity, although Vos and de La Rosa (1980) reported that food type and the female's reproductive profile or history can determine the mode of reproduction in *Artemia*. However, Sorgeloos et al. (1976) explained that very low oxygen levels of lower than 2 mg/L stimulate the formation of haemoglobin that in turn leads to change in the mode of reproduction from ovoviviparity to oviparity. Environmental parameters have also been portrayed to determine the reproductive mode; e.g. in harsh conditions, *Artemia* reproduces oviparously but in good conditions ovoviviparity takes over (Pinto Perez., 1993; Triantaphyllidis et al., 1995; Van Stappen, 1996; Clegg and Gajardo, 2009).

The dry cysts of *Artemia* are well known for their survival in harsh conditions. One of the important characteristic of the *Artemia* cysts is being hygroscopic to the surroundings, and it is advised to store them at water content less than 10% to limit the metabolic activities. Salinities above 70 g/L will not allow the cysts to hatch while very low salinities (lower than 5 g/L) will kill the nauplii shortly after hatching. Light is of utmost important to stimulate the hatching process of *Artemia* (Vos and De La Rosa, 1980).

For the nauplii to grow well they need optimal conditions such as the temperature of 28°C, salinity of sea water (35 g/L) and pH of 7. Adult *Artemia* can sustain up to between 200 to 250 g/L.

There is a lot of documentation that *Artemia* nauplii are positively photo-tactic whereas adults go away from light. Also, *Artemia* may display a swarming behaviour along the coast shores or pond trenches and one can observe different diurnal distribution of *Artemia* (Vos and De La Rosa, 1980; Persoone and Sorgeloos, 1986; Di Delupis and Rotondo, 1988, Van Stappen, 1996; Bruno et al, 2005).

2.2 *Artemia* distribution

2.2.1 General geographical distribution

A list of *Artemia* spots were reported by Artom (1922) (18 sites), Stella (1933) (28 sites), and Barigozzi (1946) (29 sites); according to Persoone and Sorgeloos (1980) the distribution of *Artemia* included 243 spots found over 48 countries in more than 80 thalassohaline and

athalassohaline habitats in five continents. In addition, Van Stappen (2002) showed an increase in the number of *Artemia* sites making a total of 353 in 1980.

Artemia cysts can be harvested from salt lakes, lagoons and solar saltworks (Persoone and Sorgeloos, 1980; Hoa, 2003) both inland and coastal worldwide (Ruiz et al., 2007). Seven sexual species and numerous parthenogenetic populations have been reported: *Artemia salina* (Mediterranean area), *Artemia urmiana* (Iran), *Artemia tibetiana* (Tibet), *Artemia sinica* (China), and *Artemia sp*. in a non-defined location in Kazakhstan (Pilla and Beardmore, 1994; Gajardo et al., 2002; Clegg and Gajardo, 2009). *Artemia franciscana* is largely spread in North, South and Central America and *A. persimilis* is found only in some parts of Argentina and Chile (Gajardo et al., 2002). Two species of *Artemia* can be found at the same saline water body; e.g. both parthenogenetic and sexual populations have been observed in Mediterranean saline sites (Vos and De La Rosa, 1980).

Artemia is unable to migrate from one saline biotope to another through the sea as it lacks anatomical defense structures against predators such as fish and crustaceans. *Artemia* is thus not continuously distributed and (Vos and De La Rosa, 1980; Persoone and Sorgeloos, 1980). Salinity was mentioned to be the most critical environmental factor controlling *Artemia* distribution (Vanhaecke et al., 1987; Kaiser et al., 2006). At 40 g/L, many carnivorous fish and other predators such as invertebrates are unable to survive (Browne and MacDonald, 1982) and pave way for *Artemia.*

The main mechanism acting in *Artemia* dispersion is wind, birds (waterfowl) and deliberately human inoculation especially in solar saltworks. Normally, cysts are attached to the feet and feathers of the birds (Green et al., 2005). Flamingos, ducks and some seagulls are thought to play a big role in direct transportation of *Artemia* cysts to different geographical, areas and also indirectly through defecation after ingesting *Artemia* cysts. The work of Green et al. (2005) and Sánchez et al. (2006) proved that *Artemi*a cysts survive the transit through the digestive system of the waterbirds without being digested by the bird's digestive enzymes. Humans have intentionally inoculated *Artemia* in solar saltworks, salt lakes and coastal salt ponds over past times (Persoone and Sorgeloos, 1980) due to the increase in need of *Artemia* cysts (Persoone and Sorgeloos, 1980; Geddes and Williams, 1987; Tackaert and Sorgeloos, 1991) and often the process involved the transfer and inoculation of exotic strains or species such as *Artemia franciscana* to sites that are naturally inhabited by other species (Persoone and Sorgeloos, 1980; Geddes and Williams, 1987; Barata et al., 1995; Hoa, 2003).

Due to the possibility of *Artemia franciscana* to replace other species occurring naturally (Van Stappen 2002) detailed studies on ecology, collection and storage of viable cysts of the naturally occurring population(s) should be considered before any introduction of *Artemia* (Hoa, 2003).

2.2.2 *Artemia* distribution on the African continent

Artemia salina is widely distributed on the African continent from Tunisia to Southern Africa, although very little is understood pertaining the distribution of *Artemia* in Sub-Sahara Africa (Van Stappen, 2002). For example, nine spots of *Artemia* for Sub-Saharan Africa were pointed out by Persoone and Sorgeloos (1980) but more spots were reported after 18 years in the review by Triantaphyllidis et al. (1998). These authors supported that for the past more than 20 years very little have been done in exploring *Artemia* distribution in Africa. The review of Kaiser et al. (2006) reported new sites in Namibia and South Africa. These authors stated that all

Artemia found in these areas are bisexual species with exception of some Namibian sites: Swakopmund and Walvis Bay Salt Pan populations are regarded as parthenogenetic while at Hentis Bay its species status has not yet been identified (Kaiser et al., 2006). Also in Kenya new *Artemia* populations were added to the list since the last general review by Triantaphyllidis et al. (1998): 3 sites with *A. franciscan*a (Kensalt saltworks, Malindi saltworks and Kurawa saltworks), 2 sites with *A. salina* (Kurawa and Fundisha) and 2 sites with unknown populations (Kaiser et al., 2006). New sites were also reported in North Africa. In total, about 127 *Artemia* populations have been recorded, on the African continent of which, only 41 populations have been determined by their mode of reproduction whereby 68% are sexual and 32% parthenogenetic (Kaiser et al., (2006).

2.3 Economic importance of *Artemia* for aquaculture

According to Lavens and Sorgeloos (2000), the development of hatcheries in the field of aquaculture led to an abrupt increase in need of live food. Sorgeloos (2001) estimated the amount of *Artemia* that had been used as larviculture live food since 1970 (few tonnes) up to the beginning of 2000s (more than 2,000 tonnes). Additionally, Sorgeloos (2009) predicted the need of *Artemia* cysts for the year 2010 to be 2,400 tonnes. The main reasons for opting to use *Artemia* as live food in larvae culture are due to its suitability in use and its promising and acceptable nutritional value for several aquatic species (Sorgeloos, 1980; Léger and Sorgeloos, 1992; Lavens and Sorgeloos, 2000; Sorgeloos et al., 1998, 2001) i.e. having a very thin carapace making it easy to be consumed by the fish larvae, a high percent of proteins (about 60% of DW), vitamins and other nutritional substrates (Sorgeloos et al., 1987).

In contrast to other live food, *Artemia* has several advantages: dry dormant *Artemia* cysts (Vos and de la Rosa, 1980) can be stored in cans for a long time and when incubated in sea water within 24 h provide live diet for larvae (Lavens and Sorgeloos, 2000). Hatched *Artemia* nauplii can be fed to reared fish and shrimp broodstocks and juveniles in the form of live, frozen or dried diet (Zmora et al., 2002). According to Sorgeloos (2009), the swimming speed of *Artemia* nauplii is not too high or too low compared to other live food, making it easy for the fish and shrimp larvae to catch and feed on them.

Many scientists documented on the determination of the nutritional content of *Artemia* based on the highly unsaturated fatty acids (HUFA) n-3 (Watanabe et al., 1980; Léger et al., 1986; Navarro, 1990; Ruiz et al., 2007). According to Léger et al. (1986) there might be variations between one batch and another of the same strain.

Therefore, bioencapsulation or enrichment has been invented by scientists for the purpose of improving the nutritional quality of *Artemia* (Dhont and Sorgeloos, 2002; Lim et al., 2003). Burton et al. (1998) pointed out the use of *Artemia* nauplii as a vehicle of spawning hormone to cure fish diseases and stimulate spawning in broodstocks while King (2002) and Kaiser et al. (2006) testified the positive use of nauplii as carrier of probiotics in marine fish larvae rearing. It was found that on-grown and adult *Artemia* outweigh the nutritional quality of newly hatched nauplii in terms of protein and amino acids contents (Léger et al., 1986; Bengtson et al., 1991; Naessens et al., 1997; Lim et al., 2001). Many experiments account for the presence of chemical substances or hormones in the biomass of adult and on-grown *Artemia* necessary for sexual maturation and improvements of fertilization rates in both fish and shrimps (Naessens et al., 1997; Wouter et al., 2002; Gandy et al., 2007).

According to Bengtson et al. (1991) before the mid 1970s commercial *Artemia* was mainly used for the pet market, and was coming from San Francisco Bay in California, USA and Great Salt Lake in Utah, USA. Lavens and Sorgeloos (2000) stated that cyst price increases in the mid 1970s were due to increased needs of fast growing hatcheries, falls in the amount of cysts harvested from GSL and bottlenecks influenced by some commercial companies. From 1980 new commercial resources emerged from both natural sites (Australia, Argentina, Canada, Colombia, France and PR China) as well as from artificial managed *Artemia* production ponds (Brazil, Thailand). During the years 1994 - 1995 the amount of cysts produced from GSL declined to a large extent leading to a short time scarcity of cysts (Sorgeloos and Van Stappen, 1995) on the market and increased price. This again prompted for exploration of other *Artemia* resources such as Lake Urmia in Iran, Lake Aibi in P.R. China, Bolshoye Yarovoyein Siberia, a number of lakes in Kazakhstan, Kara Bogaz Gol in Turkmenistan, and salt lakes in Argentina (Lavens and Sorgeloos, 2000).

However, along with improved harvesting and processing procedures, resulting in more reliable cyst quality, *Artemia* cysts from GSL have always remained prominently on the market (more than 90% of the world's cysts produced for commercial purpose) from the mid 1980s to date. It is estimated that between 80 to 85% of the total amount of *Artemia* cysts sold is being used in shrimp hatcheries while the remaining percentage is sold for feeding the larvae of marine fish and aquaria fish both in Europe and East Asia (Lavens and Sorgeloos, 2000).

2.5 Artemia production in salt ponds

2.5.1 Artemia production systems

Controlled *Artemia* production is done in coastal saltworks in which sea water salinity is increased by evaporation. *Artemia* can be cultivated in permanent and in seasonal units. Seasonal units are often referred to as artisanal saltworks and are only working during the dry season (Baert et al., 1996; Lulijwa, 2010). The ponds are only of small size (about 100 m^2) with depths ranging from 0.1 m to 0.6 m; normally they are in use a few months when the evaporation outweighs the precipitation. Anh et al. (2009) accounted for the preference of static systems in these seasonal production systems, in which the ponds are managed individually.

Permanent saltworks are much more complex systems with a number of joined evaporation ponds and crystallizers; the size of the ponds is between few to several hundreds of hectares having a depth of 0.5 m to 1.5 m. Sea water is pumped into the first pond and flows to the other evaporation ponds by gravity. In doing so, the salinity is raised due to increasing evaporation of water. *Artemia* occurs in ponds at medium salinity levels, i.e. minimum 80 g/L and maximum 140 g/L; cyst production happens in the ponds with salinity between 80 to 250 g/L (Baert et al., 1996).

Anh et al. (1997) reported that the productivity of *Artemia* biomass is affected by a number of factors such as the size of the pond, depth of water, availability of food in the culture, etc. Brands et al. (1995) reported that also the partial harvesting strategy affects biomass production. However, Brummett (2002) and Kam et al. (2008) suggested that the individual growth rate and total yield would be improved just by partial harvesting of the existing stock of fish or shrimp during the growing period as the competition will be reduced. Anh and Hoa (2004) and Anh et al. (2010) showed that partial harvesting of *Artemia* biomass over an interval

of three days provided yields higher than those of daily harvesting. Moreover Anh (2009) in her experiment on the effect of partial biomass harvest of *Artemia* where the adult *Artemia* were harvested in the interval of 1, 3, 6 and 9 days reported the total biomass yields of 1323, 1091 and 975 kg/ ha during the 3rd, 6th and 9th days respectively.

2.5.2 Pond site selection

Several parameters have to be considered when selecting the site for *Artemia* pond construction. Baert et al. (1996) listed three important parameters that are crucial for selecting the site for pond construction. One of them is climatology; the availability of enough highly saline water is essential. Normally, *Artemia* culture in ponds is done in areas where evaporation is higher than precipitation.

Another parameter is topography; it is important for the site where the *Artemia* pond is going to be constructed to be as flat as possible to simplify the work of constructing regular ponds. This will facilitate high tide inflow into the ponds, filling the pond through gravity or tidal current, and reducing the costs of filling the pond by pumping in water (Baert et al., 1996). The third parameter is the soil condition; high organic matter concentration in the pond bottom is thought to cause problems when used to construct the dikes as the earth tends to compact and reduced oxygen levels at the pond bottom may occur while the organic matter is decomposing. Acid sulphate soils are a common problem in mangrove and swampy areas as it lowers pH of water (Baert et al., 1996). Good soils like heavy clay reduce underground leakage (Baert et al., 1996) and keep the water levels optimal preventing *Artemia* escaping (Anh et al., 2009).

The use of available salt ponds in *Artemia* production is convenient if some modifications will be done, but also ponds newly constructed for the same purpose are suitable for *Artemia* inoculation. Different types of ponds are useful (such as concrete, earthen and plastic lined ponds) but due to the provision of favourable nutrient exchange from the bottom with the water high priority is given to earthen ponds. For high *Artemia* production in a pond to be reached, suspended food particles together with high nutrient levels should be given by the water coming in and for this reason salt ponds relying on intake water coming directly from the sea are not desirable (Jan, 1979).

According to Baert et al. (1996) in order to stop the development of benthic phytoplankton, to trigger the desired microalgae growth and to increase evaporation rates, the modification of the salt ponds for *Artemia* production by increasing the depth to 40-50 cm in regions with high temperatures is advisable. This is possible by digging a boundary ditch and using the dugout earth to construct the dikes. Anh et al. (2009) suggested that, good *Artemia* production ponds should range in surface area from 0.05 to 0.5 ha.

2.5.3 Pond preparation

In many sites where *Artemia* production takes place, preparation of pond begins at the end of the rainy season when the ponds are drained, scraped and then sun-dried for not more than a week. Sometimes liming can be done in case the pH is less than 8; the amount of lime applied is 10-15 kg/100 m^2 and it is applied by spreading over the pond bottom followed by drying for not more than three days. To avoid the loss of the cysts floating on the water surface by being driven by the winds and to aid harvesting by concentrating the cysts to one area (Baert et al., 1996), wind breakers such as bamboo or plastic sheets are used. These wind breakers are installed at the downwind pond corner.

2.5.4 Management of *Artemia* ponds

2.5.4.1 Importance of managing *Artemia* ponds

Day to day monitoring of the pond is needed for proper management; Anh et al. (2009) explained that in order to get a good result it is very important to properly manage the pond by making sure that the turbidity, salinity and temperature are kept optimal through taking care of water supply and pond modification. Also, suitable food supplements should be provided to guarantee faster increase of the population as described hereunder.

2.5.4.2 Water supply, exchange and removal

In *Artemia* production ponds, water inlet and outlet canals are designed to aid filling and draining the ponds (Baert et al., 1996). In most cases, green water (GW) is pumped with an interval of two days to increase the water level between 2-5 cm in order to compensate for the water lost due to seepage and evaporation, at the same time giving *Artemia* some food (Anh et al., 2009).

The water level is controlled according to turbidity levels, salinity of culture and fertilization ponds, but also in order to avoid high temperature peaks. It is documented that 1.5 to 2 months after inoculation, salinity level may be high (even above 120 g/L) and water quality problems are likely to be observed. To avoid this problem at this time, it is necessary to refresh the water in the pond between 30 to 50% to keep optimal water quality, to provide more food and to keep *Artemia* density at an optimal level (Anh, 2009).

2.5.4.3 Supplemental feeding

Fertilization is important to enhance primary production in the culture ponds. The need for nutrients by *Artemia* in salt ponds has been widely documented. According to Wear and Haslett (1987), Wurtsbaugh and Gliwicz (2001), Zmora et al. (2002) large extensive pond cultures and populations in natural habitats rely entirely on the presence of natural microorganisms as food. In small scale, semi intensive and intensive culture systems on the other hand, the food supply depends to a large extent on supplementation with agricultural and industrial food processing by-products manure or other fertilizing agents.

Inorganic nutrients (C, N, P) can enter the photo-autotrophic system if uptaken by photosynthetic algae; organic nutrients are processed through heterotrophic systems (heterotrophic bacteria) or are eaten directly by the cultured species. Not all algae are consumed by *Artemia* so control of algal composition is of utmost importance. To promote green algae (*Tetraselmis sp., Dunaliella sp.*) and diatoms (*Chaetoceros sp., Navicula sp., Nitzschia sp.*), a high N:P ratio of 10 is advisable but due to the fact that phosphorus tends to dissolve very difficultly in saline water and is deposited at the pond bottom, a ration of 3:5 is thought to be more suitable (Baert et al., 1996). Anh (2009) proposed the application of 4.7 g/m^3 of fertilizer (both urea and DAP) in the ratio of 5:1 when fertilizing the pond.

The application of supplemental feeds such as rice bran, soy bean meal etc. in the *Artemia* culture ponds was emphasized by Brands et al. (1995) and Baert et al. (1997) in order to keep algal growth at an optimal condition and making it available as natural food for *Artemia* when natural food is limited in the culture ponds. They may be introduced into the pond at a rate of 20 to 30 kg/ha/day for the first 3-6 weeks (Anh et al., 2009, Lulijwa, 2010). Likewise, manure

can be applied at a rate of 29-43 kg DW/ha/day during the first month and in the following months the application of manure is reduced to 50% (Lulijwa, 2010).

Baert et al (1996) recommended the application of 0.5 to 1.25 ton/ha of organic manure at the start of the production season by using 100 to 200 kg/ha in two to three days. For example, in Vietnam the use of 500 kg/ha of manure is commonly applied as soon as the algae levels start to decrease. It has been reported by Schroeder (1980) and Maldonado-Montiel et al. (2003) that poultry manure is the best substrate for the growth of microorganisms, mainly bacteria linked to the organic matter, paving the way to the development of high population of protozoa. In addition it has a high nitrogen and phosphorus content (Arredondo, 1993). However, the decomposition of poultry manure tends to reduce the oxygen levels in the experimental system due to its high BOD requirements (Schroeder and Hepher, 1979).

Anh et al. (2009) reported the increased production in biomass of *Artemia* with varieties of supplemental feeds. The biomass production was improved in the range of 1.79 – 2.44 ton WW /ha after 12 weeks of culture period. The type of supplemental feed that gave the most promising results was the co-feeding involving pig manure together with rice bran or soy bean. When different supplemental foods are applied they will have a positive effect on the growth and total production although they will not have an effect of the proximate composition of *Artemia* biomass. In the work of Anh et al. (2009) a 73 – 74% survival was reported after eleven days culture period in green water and pig manure (GW+PM) was supplemented with either rice bran or soya bean meal (RB or SBM) and at the same time an increased growth rate and reduced maturation period due to increased provision of food was shown. They also observed higher power of producing in large quantities; fertile individuals together with increased production of *Artemia* biomass fed with GW containing N/P ratio of 5 with satisfactory microalgae such as *Chaetoceros sp.* and *Nitzschia sp. (*Anh et al., 2009).

Food supplementation may have undesired side-effects, such as the proliferation of macro-algae. Several authors (Baert et al., 1996; 1997; Anh et al., 2009; Hoa, 2007; Lulijwa, 2010) reported that in order to control the fast growth of algal mats called 'lab-lab' and at the same time to aid in making bottom organic particles in the water column available again for *Artemia* and to favour development of beneficial algae, raking of the pond bottom should be done.

2.5.4.4 Predator control

There are several ways used to control predators in the *Artemia* culture ponds. Baert et al. (1996) suggested the use of a mixture of urea and hypochlorite (5 mg/L and after 24 h later 5 mg/L of hypochlorite), or derris root (1 kg.150 m^{-3}), rotenone (0.05 to 2.0 mg/L), tea-seed cake (15 mg/L) and dipterex (2 mg/L). Dipterex kills smaller predators such as copepods but it is also very toxic for shrimp. Another way of controlling predators is by installing screens (either filter bag of stainless steel screens) to filter intake water and retain the predators; usually filters with a mesh size less than 70 µm are used although Baert (1996) reported the efficiency of filters with 120 µm mesh size. Anh et al. (2009) and Lulijwa (2010) reported the efficiency of a 500 µm mesh filter to collect the eggs and larvae of fish.

2.5.5 Inoculation of *Artemia franciscana* in solar salt ponds

SFB *Artemia* is widely used for inoculation in tropical countries because this strain tends to display fast growth and high tolerance to temperature and salinity (Tackaert and Sorgeloos, 1991). Moreover, SFB *Artemia* produces small sized cysts, its HUFA content is high and

generally high hatching rates and hatching efficiency are the rule (Tarnchalanukit and Wongrad, 1987; Tackaert and Sorgeloos, 1991; Van Stappen, 2009). All these factors make this strain a good and promising candidate for inoculation (Tackaert and Sorgeloos, 1991). The effect of temperature differs to a large extent between one strain and another (Persoone and Sorgeloos, 1980; Vanhaecke et al., 1987). According to Baker (1966); Vanhaecke et al. (1984) higher temperatures (35°C) can cause death for *Artemia franciscana*. In relation to this, Thoeye et al. (1987) observed that *Artemia franciscan*a can survive well when put in environments with highly variable temperatures (20-35°C), but is less tolerant when exposed to environments with constant high temperature.

In laboratory tests, San Francisco Bay type *A. franciscana* has been shown to be less resistant to high temperature than Vietnam *A. franciscana*, for which the SFB strain has been used as original inoculation material (Clegg et al., 2000). Likewise, Frankenberg et al. (2000) found that, adults raised in the laboratory from Vietnam cysts showed improved thermal resistance compared to those raised from San Francisco cysts. Clegg et al. (2000) suggested that as the difference in thermal tolerance between San Francisco Bay and Vietnam strains were maintained in the second generation, the natural selection process in the Vietnamese ponds has created adults with higher thermal resistance. Bowen et al. (1978) and Persoone and Sorgeloos (1980) reported that different *Artemia* biotopes experience different water temperature and salinity and hence geographical isolation of *Artemia* populations in biotopes with variation of these parameters leads to differences in tolerance ranges.

Due to the promising results of *Artemia* inoculations done in salt fields in the Philippines (De Los Santos et al., 1980) and Thailand (Tarnchalanukit and Wongrat, 1987), *Artemia* was inoculated in Vietnam as well (Quynh and Nguyen, 1987) and in other countries.

Tackaert and Sorgeloos (1991) reported the low productivity of the parthenogenetic *Artemia* strain in Tanggu saltworks, China, and hence suggested the inoculation of San Francisco Bay *Artemia* thanks to its wide salinities and temperatures tolerance to cope with the eutrophication of the salt works and to improve the quality of the salt production in the area.

In Brazil, SFB *Artemia* from California (USA) was introduced in Macau, Rio Grande del Norte, in April 1977 (Camara and De Castro, 1983 in Camara and Tackaert, 1992; Hoa, 2003) and from here *Artemia* is believed to have dispersed by humans, birds and wind in a lot of other salterns in the area. In Vietnam, the original cyst yields of 85 kg/WW/ha/crop cysts in 1990 were followed by further success in 2009 in which more 45 kg WW/ha/crop was produced. Cyst yields have been shown to improve even exceeding the yields produced with the original SFB (Hoa, 2002). In 1990 about 1.4 tonnes of raw cysts were produced from a culture area of 16 ha (Brands et al., 1995) and in the year 2001 the production in the Mekong Delta reached 50 tonnes of raw cysts (Anh, 2009). This author showed the biomass production of between 25 to 50 kg/ha/day and suggested for more production of up to 0.7 to 1 tonnes/ha within 4 to 5 months of the culture period if the ponds are well managed. In the Ifaty salt works in Madagascar, where water temperature can reach up to 37°C and water depth is always less than 25 cm, Vinh Chau *Artemia* (Vietnam) has been inoculated in 1992, 36.71 kg cysts DW/ha/ month were harvested over an eight months culture period. Vanhaecke et al. (1984) and Vanhaecke and Sorgeloos (1989) proposed that for a better selection of an inoculation strain it is important to rely on available data on temperature and salinity tolerance for growth and production performance. There is a need to consider to what extent and how fast the introduced strain can cope with the ambient conditions (Vos and Tansutapanit, 1979).

3 MATERIALS AND METHODS

3.1 Field experimental work

3.1.1 Site description

The study was conducted in one of the villages in Malindi, Kenya, known as Kadzuhoni. 'Kadzuhoni' is a Giriama word meaning very small river (personal communication with the community). This village is situated in the coast region, county of Malindi, Magarini district and in the small town called Gongoni, located at 3° 50' 0" South, 39° 46' 0" East (Google map, 2011) as shown in Figure 3.1. The inhabitants of that area are Giriama tribes' people (Facts and Figures, 2007).

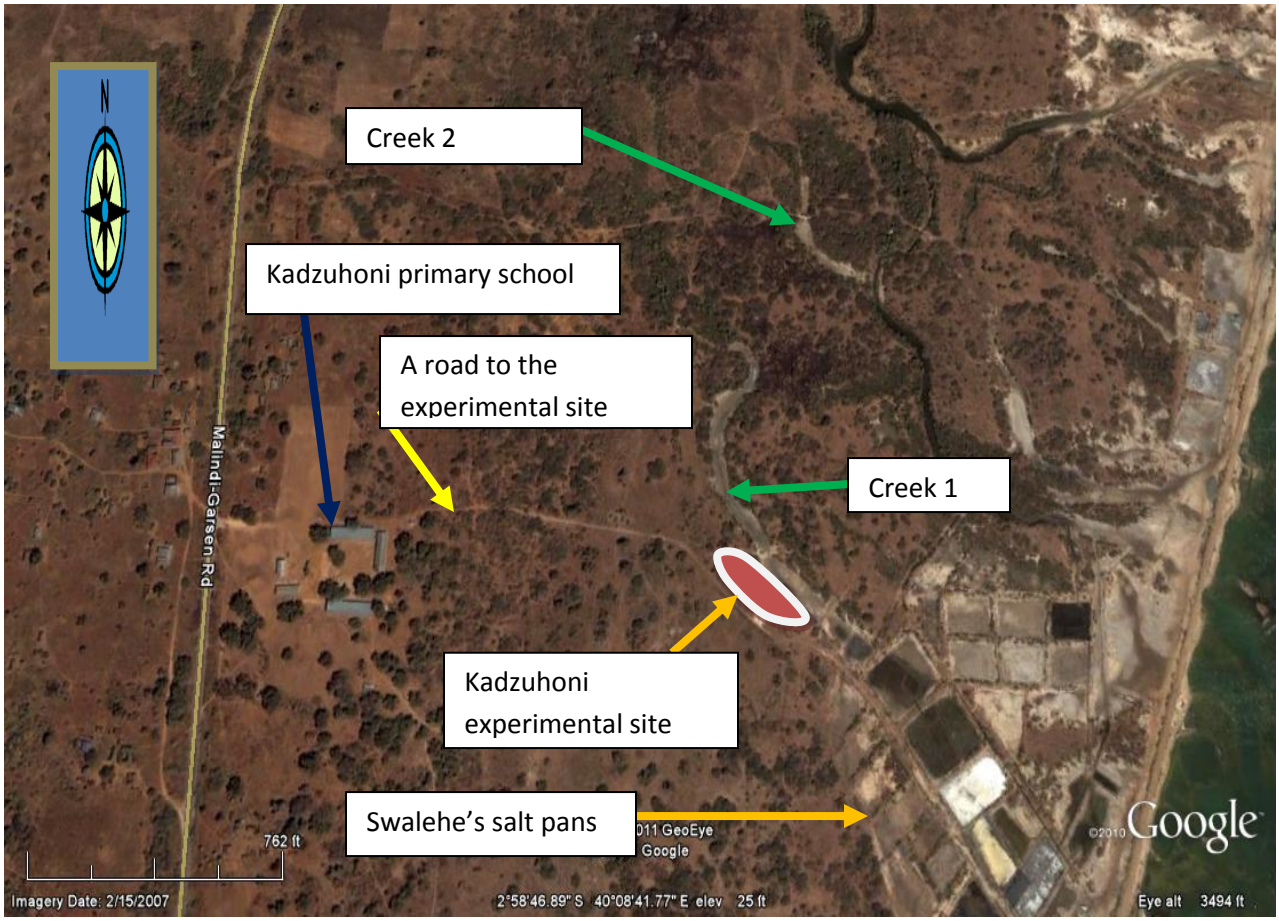


Figure 3. 1 Gongoni small town with location of Kadzuhoni experimental ponds site

According to the Kenya National Bureau of Statistics, the population of Malindi as per 2009 census was 68,311 people. The project site is about 120 km from Mombasa City in the direction of Lamu (Kenya Roads Agency, 2009). The climatic condition along the coast is tropical (Kenya meteorological department, 2011) with maximum air temperature 36°C and the average temperature 27°C (Figure 3.2). Normally, the area is characterized by two dry seasons (Figure 3.2): a long dry season that starts between July to September and the short dry season that occurs between late January to March (KMFRI researchers and Gongoni personnel, personal communications; Africaguide.com, 2011). On the other hand, most parts of the country including the coastal areas experience two rainy seasons (Figure 3.2), the long rains falling around April to June, and short rains between October and December. The coastal areas have

an average of 1,016 mm of rainfall per year (KMFRI researchers and Gongoni personnel, personal communications; Africaguide.com, 2011).

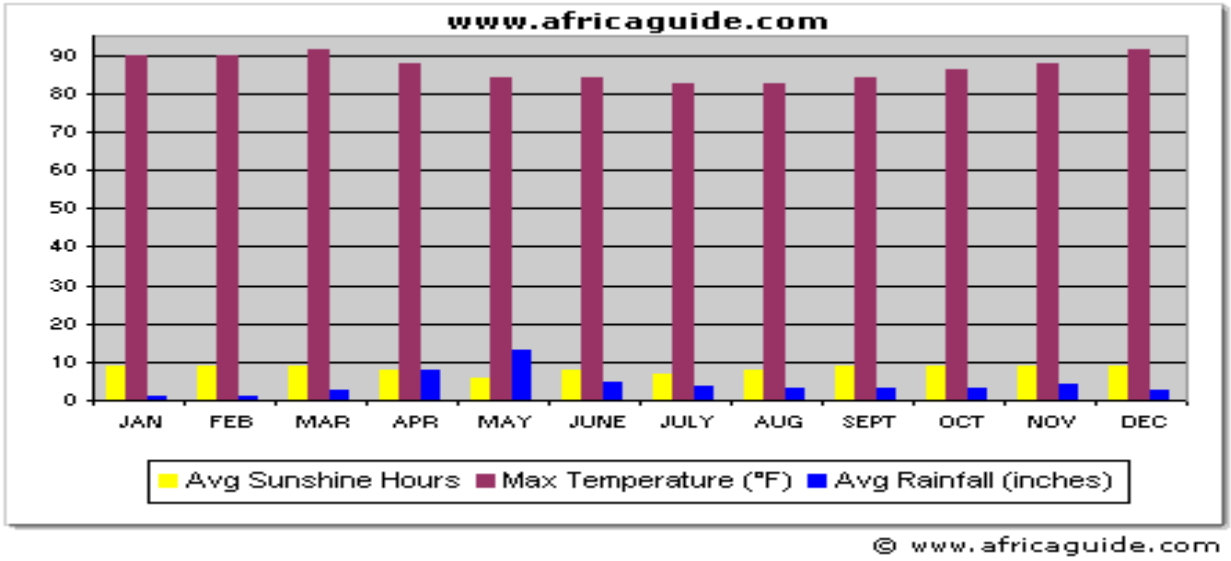


Figure 3. 2 Climatic conditions for Mombasa - Kenya, adopted from africaguide.com (2011)

The area is characterized by a sandy soil, receiving two high and low tides regimes per day and one spring tide per month (Kenya meteorological department, 2009; KMFRI, personal communication). Apart from tourism, the main economic activities are fisheries and salt production. Magarini district is surrounded by eight big salt companies: Kensalt Limited (which is close to the project site), Krystalline Gongoni, Fundisha, Kurawa Industries Limited, Malindi saltworks, Kemusalt, Krystalline Marereni, Tana salt and Mnarani salt farm; all of them are operational (Nyonje, unpublished report). According to Bossier et al. (2010), the industries employ between 100 - 150 workers each on a regular basis and about 400 people at peak moments.

3.1.2 Experimental design

This study involved two types of feeding regime: fertilized green water and non-fertilized green water (fertilized culture ponds and control culture ponds) to observe if there was any effect on growth, reproduction, and cyst production on *Artemia* in the culture ponds. There were two creeks that were supplying water into the field site; the creek 2 (average salinity 45 g/L) was supplying water to creek 1 (45 to 65 g/L salinity). Creek 2 comes from the sea and feeds creek 1 before it empties its water back to the sea. Nearly at the mid-point of creek 2, creek 1 receives water and transports it to the artisanal salt farms (Figure 3.1).

The experimental ponds were constructed at Swalehe's farm. A big pump was pumping water from creek 1 (Figure 3.3) into the reservoir ponds (R1 & R2). A small pump was used to pump fertilized green water or non-fertilized green water into the respective experimental ponds. The small pump was also used to pump water into the evaporation ponds (A1-A3) to build up the salinities before the water from these ponds was used to raise the water levels in the culture ponds ponds. When water level in creek 2 went down and it could not supply creek 1 with water, the small pump was used to pump water from creek 2 into creek 1.

There was a reservoir which was fertilized (R1) and a reservoir which was not fertilized (R2). In both we were trying to produce green water. The water of each reservoir was pumped to their

corresponding three replicate *Artemia* production ponds. Due to practical problems (invasion with predators) we had to re-do the control and so on infact the experiment has been split over time.

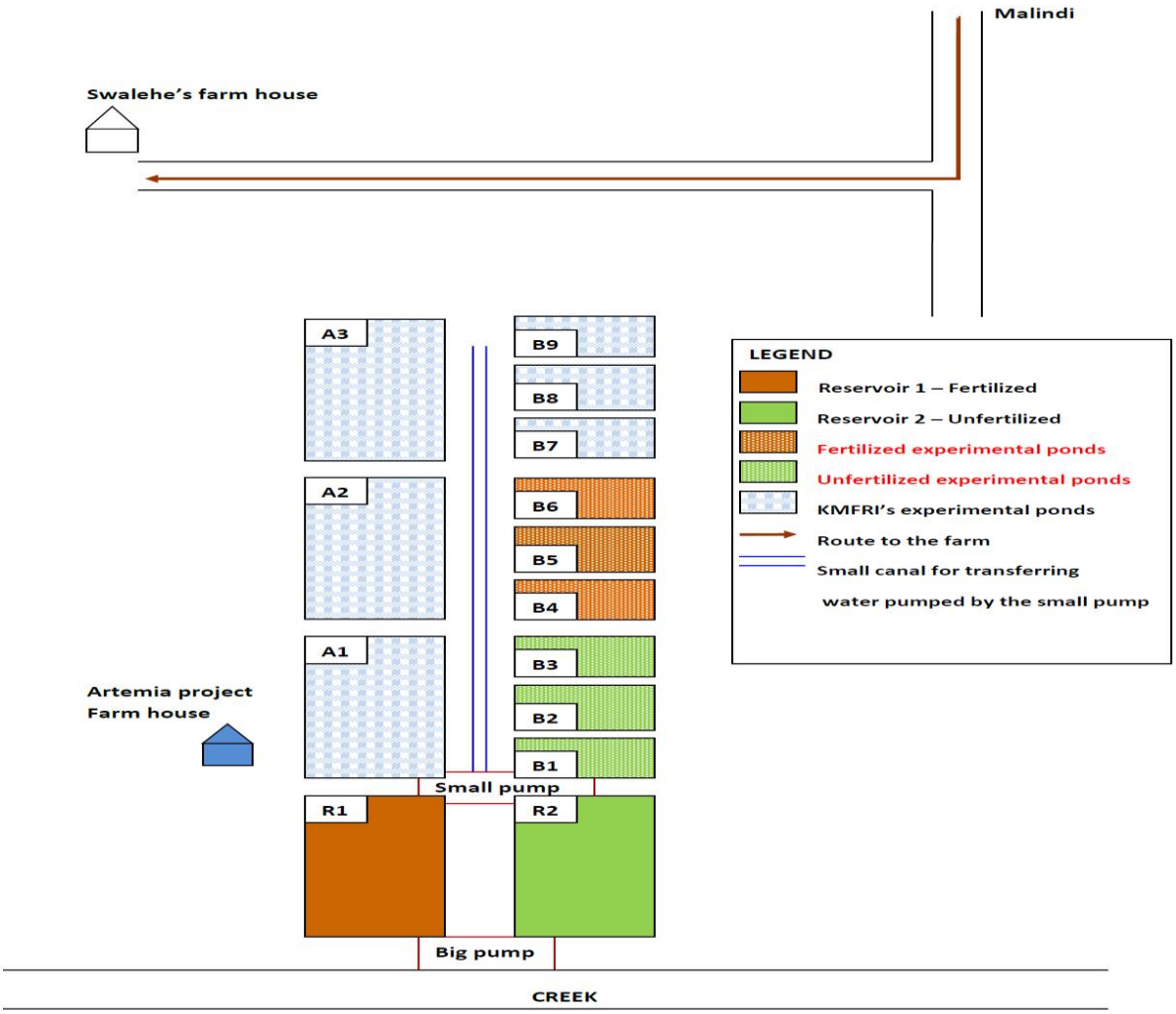


Figure 3. 3 Schematic layout of the Kadzuhoni's experimental project site, with 3 replicate control and fertilized ponds B1 to B6.

The first run of the experiment lasted for 30 days (fertilized treatment). Likewise, the second run (control) took 30 days and there was an overlap of both experiments on the 27th day of the first run after inoculation.

3.1.3 Preparation of the ponds

All six experimental ponds used were newly constructed and each pond had an average area of 300 m^2 (15 m x 20 m). Amongst these experimental ponds, only one control pond (B3) and one fertilized pond (B6) had trenches of about 1 m wide and 0.4 m deep. These trenches were being used as a refuge for *Artemia* when the temperature becomes too high. Liming was not done in any of the six experimental ponds.

Control culture ponds (B1, B2 and B3) were drained and dried for 7 days. Then water was filled at 2 cm level of the platform and disinfection was done with a combination of urea and sodium

hypochlorite solution in the ratio of 5 mg/L urea, and 24 h later the same amount of hypochlorite was applied to kill any fish or predator that could be still surviving in the pond as recommended by Anh (personal communication). Following the application of these chemicals, the ponds were left for few days to pave the way for the chemicals to evaporate and degrade. After preparation, highly saline water of above 100 g/L from the reservoir ponds was filtered through a 400 µm mesh to remove any fish or other predators. A second filter of about 70 – 80 µm was set on the pipe distributing water from the channel to the culture ponds to ensure maximum elimination of all predators that could pass through the first filter. In this way the water level at the platform of the fertilized culture ponds was filled up to 25 cm before inoculating them. The control ponds were filled up to 15 cm level before inoculation and after inoculation water level was raised slowly as the experiment was running up to 22 cm level.

For the fertilized culture ponds, the same procedures for draining, drying the ponds, pumping and filtering water was applied. The differences were that no disinfection was done in the fertilized culture ponds and the water level before inoculation was raised up to 30 cm. On the tenth day of inoculation, wind breakers were installed into all the fertilized culture ponds at the opposite side of the wind direction (Figure 3.4).

Figure 3. 4 Installation of wind breakers into the culture ponds

During the second week after inoculation, flags made up of polyethylene bags and clothes were installed at the sides of the ponds to scare birds that were feeding on *Artemia.*

3.1.4 Management of fertilization reservoir pond

The types of inorganic fertilizer used in the fertilization reservoir pond were urea and diammonium phosphate (DAP) (N-P-K of 18-46-0) and the application rate for both was 5.7

g/m^3 in the ratio of N:P = 5:1 as suggested by Anh et al. (2009). Before being applied to the fertilization pond, fertilizers were dissolved in a 20 L plastic bucket with sea water; diammonium phosphate (DAP) was the first to be applied followed by urea. Fertilizer was added weekly or when the water was transparent and this was normally done between 9 to 11 am and when the sky was not cloudy. Weekly poultry manure was put into 2 bags each with approximately 40 kg, placed at the windward side of the pond, and then punctured to allow slow release of the nutrients. Following application of fertilizers, algal proliferation was observed after 2 to 3 days and after filtration (see above) the algae were supplied to the *Artemia* ponds by pumping.

3.1.5 *Artemia* inoculation

Artemia franciscana VC strain cysts (30 g) produced by the College of Aquaculture and Fisheries, Can Tho University, Vietnam, was hatched into a 20 L plastic bucket filled with filtered sea water of 35 g/L at a density of 2 g cysts/L. Constant aeration was provided from the bottom of the bucket, illumination was provided by four fluorescent tubes, and ambient temperature was at 26 - 28°C (Van Stappen, 1996).

Cysts incubation was done at KMFRI Mombasa around 2 pm and left overnight; then freshly hatched nauplii were packed into plastic bags (two bags, one inside the other) ¾ filled with air (Figure 3.5) and transported to Malindi - Gongoni. In the late evening at 5 pm the plastic bags with nauplii were placed into the pond for some time so as to acclimate the nauplii to the new environmental conditions. Inoculation was done at a density of 100 nauplii/L and at the windward side of the pond (Anh et al., 2009).

Figure 3. 5 Incubation/hatching of *Artemia* cysts and hatched nauplii packaging in polyethylene bags ready for transport to the experimental culture ponds.

3.1.6 Pond management

Pond management followed the procedure described by Anh et al (2009) and involved daily raking (Figure 3.6). In the first four days after inoculation, water lost due to evaporation and seepage was compensated by daily pumping of highly saline water from evaporation ponds (A1,

A2 and A3 and sometimes B7, B8 and B9) to raise the water level with 2-5 cm in the morning and/or evening.

Figure 3. 6 Raking the pond bottom

From the fifth day after inoculation, GW from fertilized reservoir R1 and non-fertilized reservoir R2 was pumped to the culture ponds B4, B5, B6 and B1, B2, B3 respectively.

3.1.7 Data collection and analysis

3.1.7. 1 Creeks and reservoir ponds

Temperature and salinity were measured daily at 8 am, 2 pm and 6 pm using a mercury thermometer and a hand refractometer (Model ORD/ATC-WZ-201/211 0 – 100 g/L) respectively. Water level was monitored using a meter rule installed into the pond close to the dike; turbidity was measured daily at 8 am and 2 pm using a Secchi disk. In addition, samples for nutrients, phytoplankton and zooplankton were taken monthly (1st month, before *Artemia* inoculation, 2nd month - treatment run, 3rd month-control run) as explained below.

Phytoplankton samples were taken from creek 1 and creek 2 and the reservoir ponds R1 and R2. Approximately 20 L of water was filtered using a 200 µm mesh net, the samples were fixated with lugol solution and immediately transported to KMFRI – Mombasa laboratory for analysis. In the laboratory, 50 mL of sample was concentrated by reducing the volume of water and 5 drops (equivalent to 0.02 mL each) per sample was poured on a slide using a dropper. Counting of the phytoplankton cells was done under the microscope (Inverted microscope LEICA DMIL 520804). Identification was done according to literature procedures (Hailegraeft et

al., 2003; Carmelo R.T., 1997; Boney A.D., 1975; Botes L., 2003). Phytoplankton abundance per pond was calculated using the formula:

Abundance (in total number of cells/L) =

((∑ Total number of phytoplankton cells per sample)*concentrated

volume/volume viewed)/ volume filtered

Concentrated volume = 50 mL

Viewed volume = 0.1 mL

For zooplankton sampling a known volume of water (100 – 400 L) was filtered using a cone-shaped nylon net with 332 µm mesh size using 4 replicates. The collected samples were poured in formalin (5%) and transported to KMFRI, Mombasa laboratory for analysis. In the laboratory, the samples were rinsed with tap water, put in a petridish with some water and identification and counting of the samples was done. At high zooplankton concentration a subsample of 10 mL was taken and counted according to literature identification keys (Dhargalkar and Verlecar., 2004).

Monthly samples for phosphate, nitrate and ammonia were collected between 9 and 11 am. Three different points in each pond were selected where water was drawn using a 1L beaker and filtered over a 118 µm mesh net. A sub-sample from this beaker was poured into a 120 mL bottle, stored on ice and immediately transported to Mombasa KMFRI laboratory for analysis.

Determination of phosphate–P was done essentially according to the calorimetric method by Murphy and Riley (1962), which is based on the formation of the highly coloured blue phosphoromolybdate complex, modified according to Koroleff (1983).

Determination of ammonium-N followed the method of Grasshoff and Johansen (1973) and Koroleff (1983), based on the formation of the blue coloured indophenol complex by phenol and hypochlorite in the presence of the NH_4 and NH_3.

Nitrate–N was determined using the method based on reduction of nitrate to nitrite which is then determined colorimetrically via the formation of an azodye, based on a heterogeneous reaction with copper-coated cadmium granules (Grasshoff, 1983).

3.1.7.2 *Artemia* culture ponds

3.1.7.2.1 Monitoring and analysis of physico-chemical parameters

The procedures for salinity, turbidity, nutrient parameters (phosphate, nitrate and ammonia) and planktons (phytoplankton and zooplankton) were the same as for the creeks and reservoir ponds.

3.1.7.2.2 Monitoring of *Artemia* population

Basically sampling was done weekly for population density, population composition, biomass estimation (as from 7th day) between 9 – 11 am, according to the procedure described below.

Population density

Ten sampling points, consisting of the 4 corners, 2 points at the middle of the pond, 2 points at the midway of the pond length and 2 points at the midway of the width were chosen. Before sampling, manual stirring of water was performed in order to equally distribute the animals in

the pond and a sample was drawn using a 1 L glass beaker. Then the 10 samples collected were pooled and mixed into a 15 L plastic bucket, from which 1 L was drawn and filtered over 118 µm and counted.

Population composition

The same procedure for population density was applied, but nauplii, juveniles and adults were counted separately. To achieve this, 118 µm, 500 µm and 1 mm sieves were used to retain the nauplii, juveniles and adults respectively in a liter of sub-sample from a pooled sample (see above). The number of nauplii, juveniles and adults retained were counted.

Growth

To measure the growth of *Artemia*, 30 animals were taken from a pooled sample from each pond (see above) and stored into a 120 mL bottle, quarterly filled with alcohol (80%) before transport to the KMFRI laboratory in Mombasa for analysis. Total length from the tip of the head till the end of the telson was measured using a micrometer (0.01 x 0.25 mm). To determine the dry weight of *Artemia* biomass, only the samples for day 7, 14, 21 and 28 were taken into account. The total numbers of animals used for determining population density were kept into a 120 mL bottle, 80% alcohol was added and the bottles were transported to KMFRI laboratory in Mombasa at the end of the experiment. The individual dry weight was determined after oven drying at 50°C for 22 h using a balance CITIZEN: CY360 (accuracy 1 mg).

Maturity

At day 14, the number of females with fully developed broodsac was counted under the dissecting microscope (LEICA) M3C1 and expressed as percentage of 30 females per replicate. The animals were taken from the pooled sample as explained above.

Fecundity

Fecundity was determined by dissecting the broodsac of 30 females with fully developed broodsac, randomly taken from the pooled sample in weeks 2, 3 and 4 (see above), and counting the offspring under the dissecting microscope (LEICA M3C1). The percentage of oviparously reproducing animals was also recorded.

3.1.7.2.3 Cyst production estimation

Estimation of the cysts released started in the fourth week as *Artemia* started to produce cysts (cyst production started before the 4th week but in very little amounts that could not be collected). Every two days in the evening, cysts were harvested from the ponds using a 150 µm scoop net and put into a 3 L plastic bucket, washed and processed the following morning. Large debris and sand was removed by washing the raw cyst product successively over a 500 µm and 250 µm mesh, and cysts were collected on a 150 µm mesh.

The primarily processed cysts were then weighed using a 5 kg weighing scale OHAUS CS Series CS5000 (accuracy 1 g), and the cysts were then stored in brine before transport to KMFRI laboratory in Mombasa, where secondary processing was done: cysts were washed with fresh water and empty cyst shells were removed by density in freshwater (Baert et al., 1996). Cyst dry weight was determined following the same procedures as described above, but drying was done at 40°C for 4 h whereby the cysts were further cleaned with fresh water to remove the salt content followed by density separation.

3.7.1.3 Statistical analysis

Data for physico-chemical parameters (temperature, salinity and turbidity), population density, population composition, maturity, total length, DW, reproductive parameters including fecundity and cyst production were subjected to Un-paired t-test to detect the effect of the treatment. Data for sexual maturity were normalized through an arcin transformation prior to statistical analysis. However, data for nutrients were subjected to one-way ANOVA to detect the effect of the treatment. To establish the effect of time and of treatment (fertilization), phosphate, nitrate and ammonia, total length, DW, fecundity, population density and cyst yield were treated using two-way ANOVA. Bonferroni's pairwise multiple comparison was used to identify significant differences between experimental sample means at a significance level of $p<0.05$. All statistical tests were conducted at 5% level of significance, using Graphpad Prism, version 5 software.

3.2 Socio-economic study

Questionnaires including open and closed ended questions were prepared with the assistance of two socio-economic experts from the department of Socio-economic programme, KMFRI laboratory, Mombasa and submitted to the local village community where the study was being conducted, to assess the success of the project in terms of potential for improvement of the living standard. The questionnaires included questions on income and daily expenditure, education level, number of family members and food security, but also on general awareness, and appreciation (see appendix). About 41 villagers were visited and interviewed at their own homes.

3.3 Laboratory tests

3.3.1 Effect of temperature on growth, survival and reproduction

3.3.1.1 Experimental set up

The culture experiment was run with two Kenyan samples (one sampled about a decade after first inoculation in Kenya, a second one about 15 years later); San Francisco Bay (SFB) and Vinh Chau (VC) *Artemia* were used for control (Table 3. 1).

Table 3. 1 *Artemia* samples used, their year of collection, ARC cyst bank number and abbreviations used in this experiment

Geographical origin of cysts	Year of collection	Cyst bank number	Abbreviation used
San Francisco Bay (USA)	2003	1574	SFB
Vinh Chau (Vietnam)	2009	1742	VC
Kensalt saltworks (Kenya)	2010	1762	Kn1
Kensalt Saltworks (Kenya)	1996	1439	Kn2

Artemia cysts of each strain were separately hatched in a 1 L glass bottle, under optimal conditions of temperature (28°C) and salinity (35 g/L) in 0.8 L of water. The cysts were incubated for 24 h and freshly hatched instar I nauplii were harvested and distributed into 0.25 L erlenmeyer flasks for culture. The culture medium was set at three different temperatures

(28, 32 and 36°C) in 80 g/L artificial saline water in different water baths, thermo-controlled by submergible heaters. In order to achieve the required salinity, Instant Ocean salt was added into de-ionized water until it reached the desired salinity. Light intensity was the same in all 3 treatments and the animals were exposed to 16 h light and 8 h dark; point aeration was also set at the bottom of each flask (Figure 3. 7).

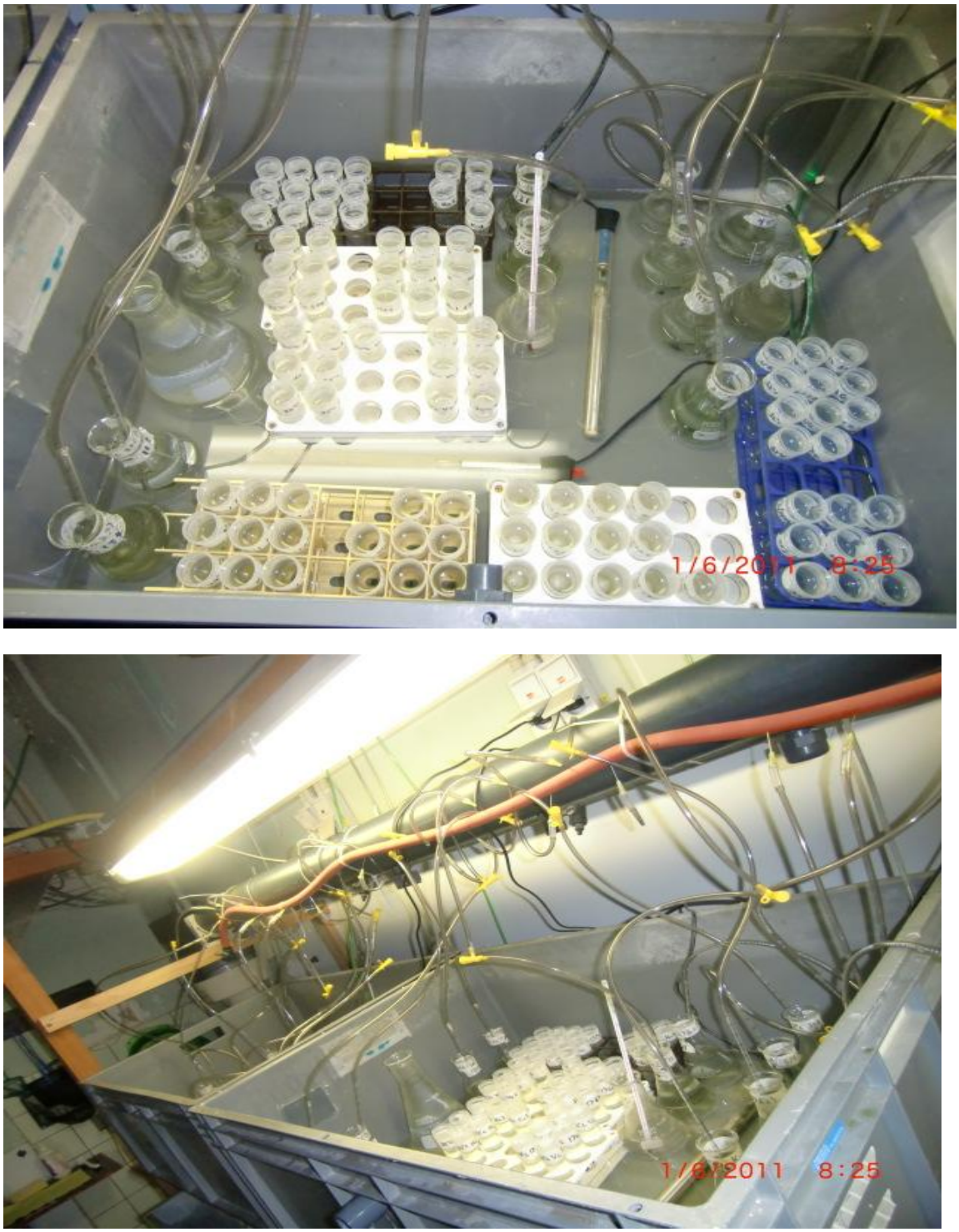


Figure 3. 7 Experimental set up showing falcon tubes and erlenmeyers immersed in a water bath, aerators and light tubes installed

Three hundred instar I nauplii were introduced into 250 mL erlenmeyer flasks in three replicates for each of the three treatments per population. The nauplii were immediately brought in the higher temperature. The flasks with nauplii were then randomly distributed into their respective water baths. When the animals started coupling 10 individual couples were placed in 50 mL falcon tubes filled up to 40 mL level with 80 g/L Instant Ocean saline water and kept at the appropriate temperature. If a male died it was replaced, if the female did the replicate was discarded. The experiment was terminated after a 45 days culture period.

3.3.1.2 Culture maintenance

The animals were daily fed with *Dunaliella tertiolecta*, after changing the water and sampling (if any), using the feeding schedule recommended by Coutteau et al. (1992) and Vanhaecke et al. (1984) (Table 3. 2). Complete water renewal was done two times over the entire culture period and the water lost due to evaporation was compensated by daily filling the flasks up to their usual 250 mL level. Complete water exchange for the falcon tubes was done on a daily basis from the day the couples were stocked.

Table 3. 2 Feeding schedule for 300 *Artemia* nauplii for the different culture temperatures (x 10^6 cells *of Dunaliella tertiolecta*) (Coutteau et al., 1992; Vanhaecke et al., 1984)

	Culture temperature		
Day	**28°C**	**32°C**	**36°C**
1	1.22	1.57	1.57
2, 3, 4	2.65	3.40	3.40
5, 6	3.87	4.96	4.96
7	5.10	6.53	6.53
8	6.32	8.10	8.10
9	10.58	13.57	13.57
10, 11	12.04	15.43	15.43
12, 13	15.16	19.44	19.44
14, 15	18.10	23.20	23.20
16, 17	18.91	24.24	24.24
18, 19	21.02	26.95	26.95
20 onwards	23.14	29.66	29.66

3.3.1.3 Data collection and analysis

Survival

Survival was determined every 4 days by counting the number of remaining live animals in the flasks.

Length

Length measurement was done from the fourth day and then repeated every 4 days. This involved randomly taking 10 animals from each flask, fixating with lugol, and measuring total body length from the tip of the head up to the end of the telson using a dissecting microscope. Later on, data were analyzed with the aid of a digitizer (KD 4300, Graphtec corp., Japan).

Reproduction

Reproduction was monitored from the first day of coupling until the female's death. This involved daily counting of the cysts and/or nauplii released per female. The data collected were used to determine the pre-reproductive and reproductive periods, total offspring per female, offspring per female per day, total number of broods per female, oviparous brood, percent offspring encysted, brood interval in days and ovoviviparous broods per temperature and per strain.

Statistical analysis

Normalization of the data of survival and percentage oviparity was done through an arcsin transformation before statistical treatment. A two-way ANOVA test (Graphpad prism, version 5) was used to detect significant interactions between strain and temperature. For all treatments, results were analyzed using one-way ANOVA to find the overall effect of the treatment and strain. Turkey's pairwise multiple comparisons was used to detect significant differences between the experimental sample means at a significance level of $p<0.05$.

3.3.2 Thermo-tolerance test

The same four samples, as used for the culture test were used for the thermo-tolerance test based on the procedure of Clegg et al. (2000, 2001) one gram of cysts of each sample was incubated in a 1 L glass bottle, filled up to 0.5 L with sea water and at 28°C. Luminescent fluorescence light was provided, and aeration was provided at the bottom.

After 24 h, freshly hatched 30 instar I nauplii were counted and placed in 40 mL glass tubes, filled with autoclaved 30 mL of 35 g/L in a water bath, set at 35°C; for each population there were 5 replicates. Following 1 h, the tubes containing the nauplii were placed in a water bath set at 28°C for 1 h to acclimatize them. The tubes were then placed back again in a water bath of 38°C for 30 min after which the tubes were removed and placed in 28°C for 3 h to acclimatize them again. The tubes were then placed in 40°C water bath for 15 min, removed and placed in 28°C water. Two h after the last heat shock, *Artemia* nauplii were fed with 87 µl of autoclaved LVS III bacteria/tube with a density of 1 x 10^7/mL (30 mL glass tube), re-capped and then placed on an electric rotator (Figure 3.8).

Survival was recorded 43 h after the last heat shock by counting the number of live *Artemia* nauplii per replicate. There was no control in this experiment.

Figure 3. 8 *Artemia* heat shock experimental set-up. Left: heat shocking of *Artemia* in a water basin; right: glass tubes on rotator

Data for survival were subjected to one-way ANOVA to detect the effect of the strain after arcsin transformation. Turkey's pairwise multiple comparison was used to detect significant differences between the treatments at a significance level of $p<0.05$.

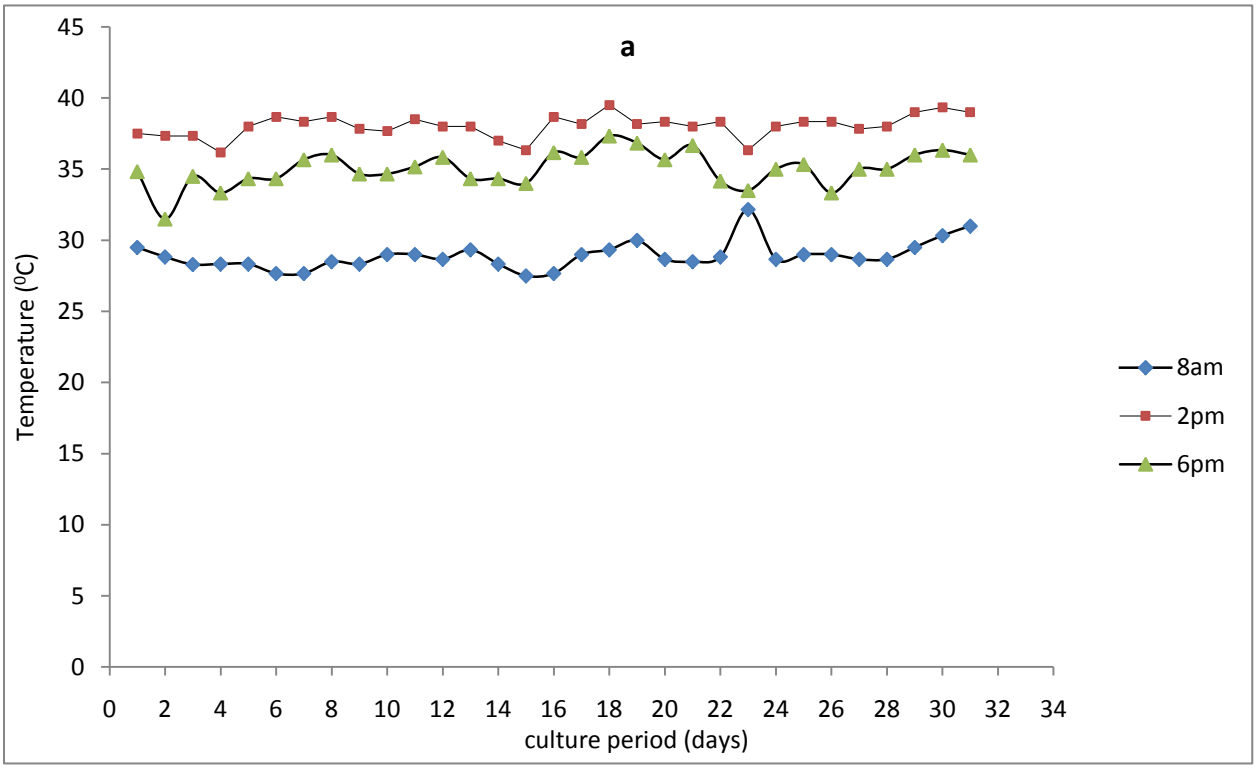
4 RESULTS

4.1 Field work

4.1.1 Physico-chemical parameters

During the day, daily temperature fluctuated in a range of 27.5 – 32.2°C at 8 am, 36.2 – 39.5°C at 2 pm and 31.5 – 37.3°C at 6 pm for the fertilized treatment, and 26.7 – 31.2°C at 8 am, 36.7 – 40.7°C at 2 pm and 33.8 – 38.0°C at 6 pm for the control. In the fertilized treatment, the highest temperatures observed in the morning, afternoon and evening were 32.2, 39.5 and 37.3°C, while 31.2, 40.7 and 38.0°C were recorded at the same time in the control (Figure 4.1a and b). Generally, temperature differences ranged between 3.3°C - 9.7°C in the first week, 2.1°C - 8.7°C in the second week, 2.8°C- 8.7°C in the third week, and 8.4°C - 2.7°C in the fourth week for both control and fertilized treatment.

The unpaired t-test did not reveal any significant temperature difference at 2 pm between control and fertilized treatment (p=0.619). A significantly higher temperature was observed in the control than in the fertilized treatment, at 6 pm in the first three weeks (p = 0.0310) and at 8 am (p=0.0164) throughout the culture period.



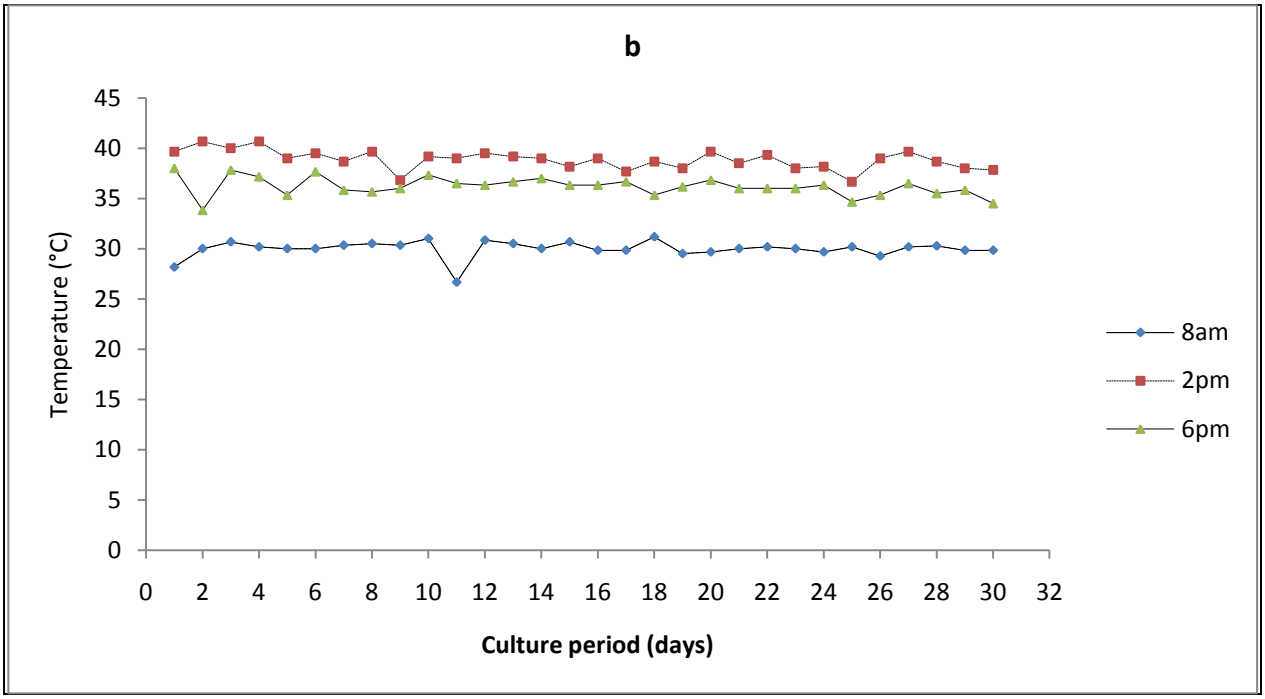
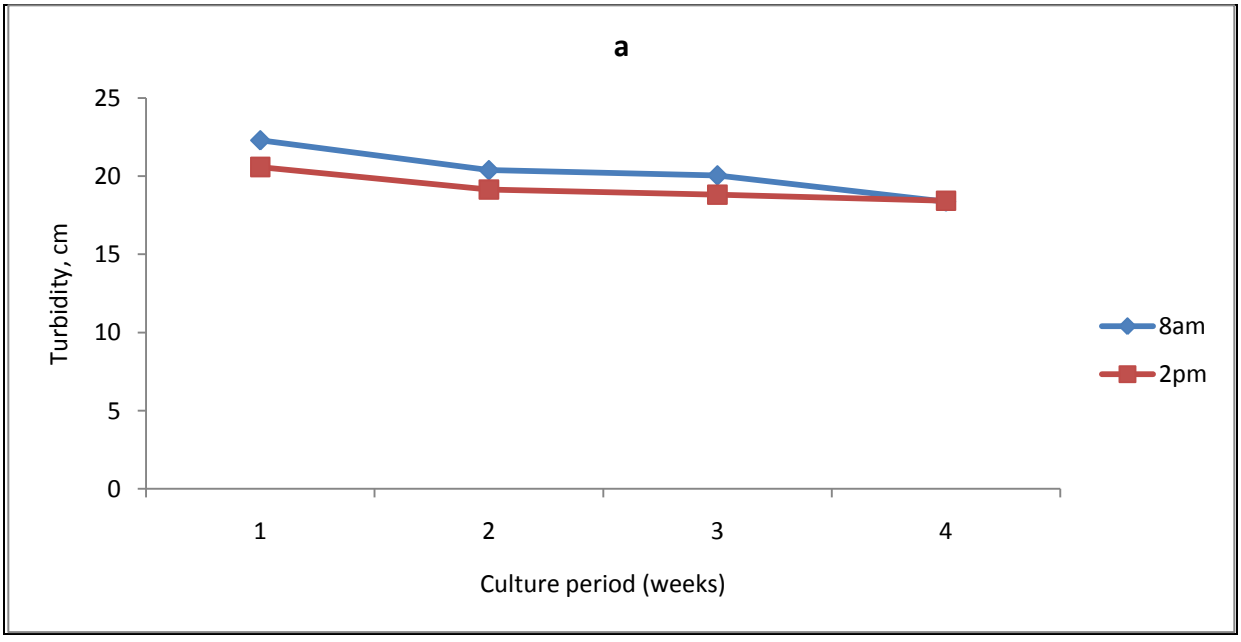


Figure 4. 1a and b Mean daily water temperature fluctuation of the fertilized culture ponds (a) and control culture ponds (b) over the culture period; points are averages of 3 observations

Mean weekly turbidity ranged from 18 cm to 22 cm for the whole culture period (Figure 4.2). The maximum turbidity level reached was 22 cm in the third week of the control run. The lowest turbidity was recorded in control and fertilized treatment during the first and fourth week respectively (Table 4.1). However, an un-paired sample t-test did not find any significant (p=0.5098) difference in turbidity between the control and the fertilized ponds for the entire culture period.



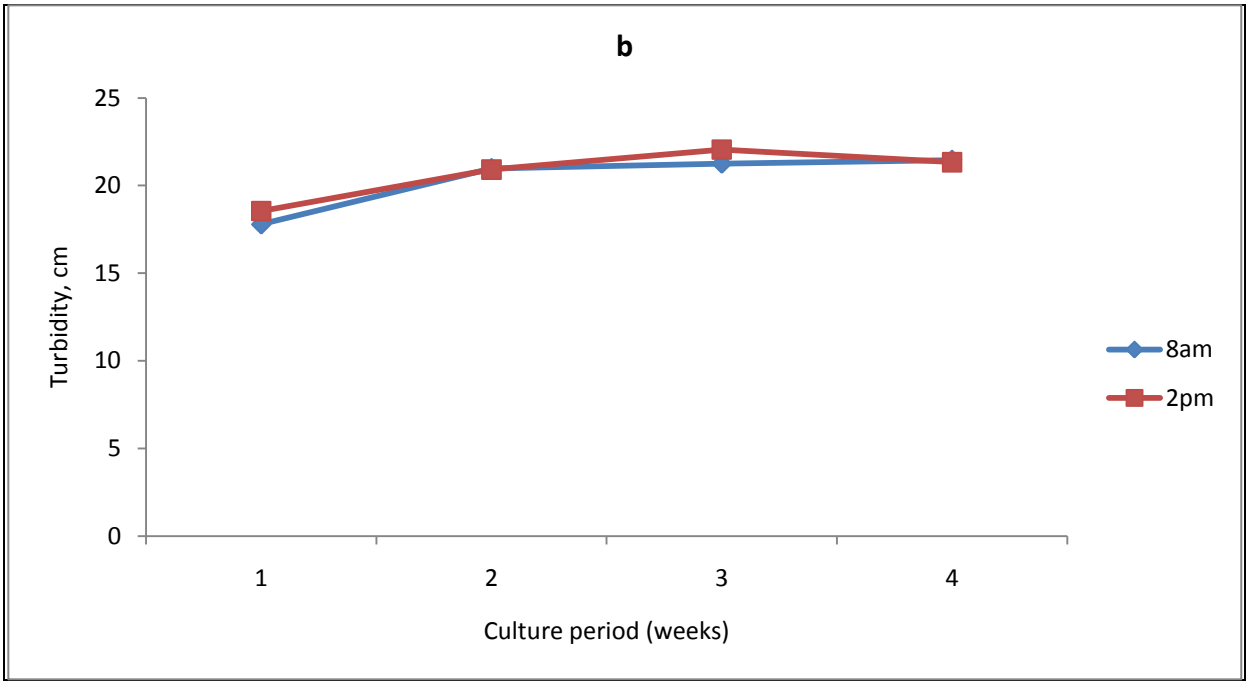
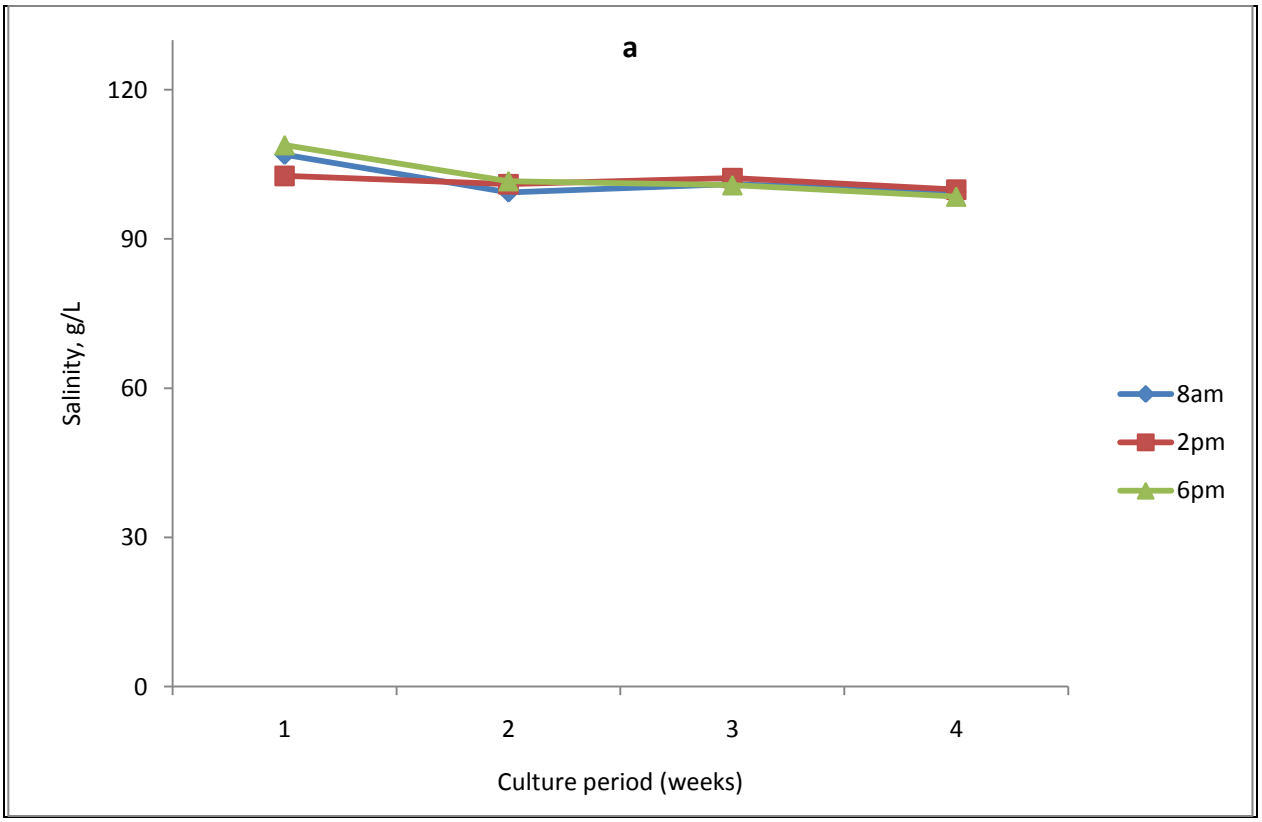


Figure 4. 2a and b Mean weekly turbidity fluctuation of the fertilized culture ponds (a) and control culture ponds (b) over the culture period; points are averages of 3 observations

Mean salinity varied from 99 to 119 g/L; the highest record was in the first week for both control culture ponds and fertilized culture ponds (104 g/L and 119 g/L respectively) (Table 4.1 and Figure 4.3). Mean weekly salinity was significantly higher ($p<0.05$) in the control than in the fertilized culture ponds for the entire culture period.



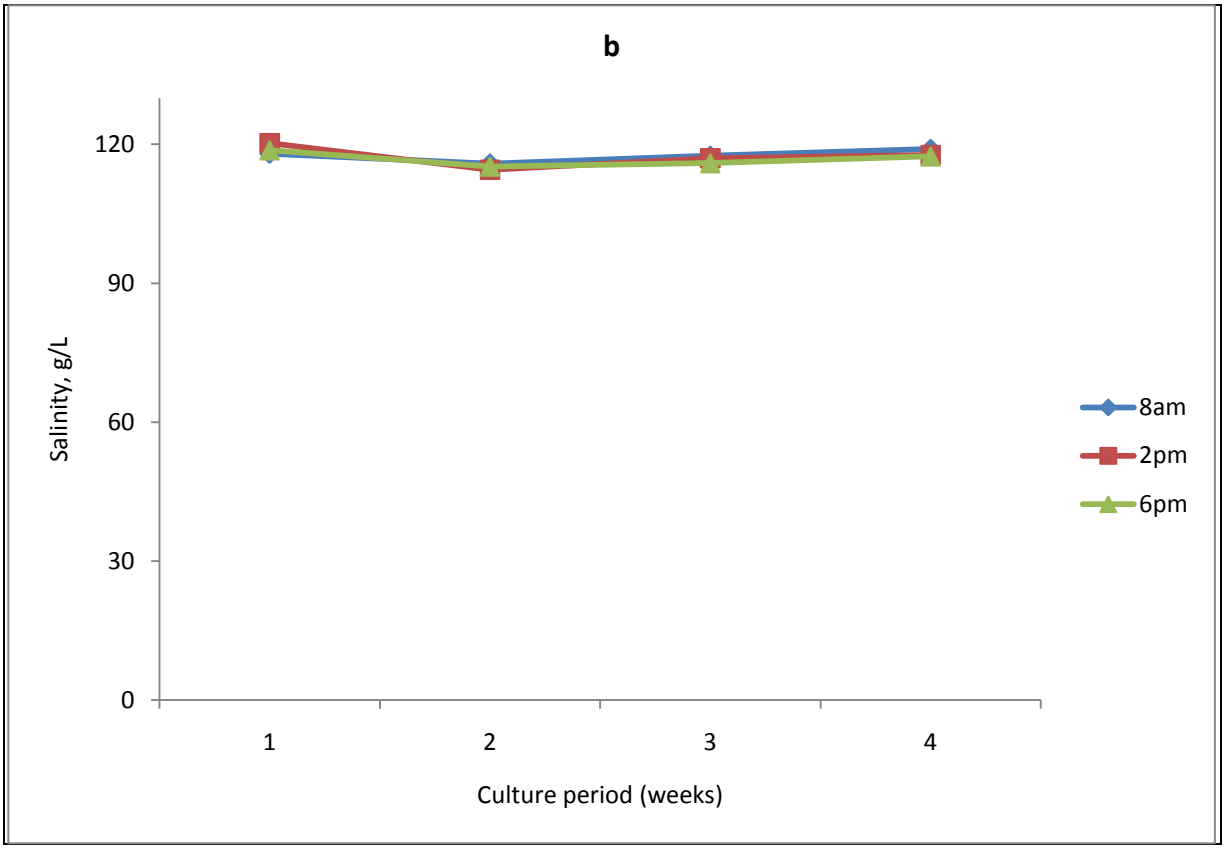


Figure 4. 3a and b Mean weekly salinity fluctuations of the fertilized culture ponds (a) and control culture ponds (b) over the culture period, points are averages of 3 observations

Table 4. 1 Mean weekly abiotic parameters (mean ± StDev. of 21 observations) of the control and fertilized culture ponds over the entire culture period

Temperature, °C, 8 am	**Fertilized treatment**	**Control**
Week 1	28.4±0.8[a]	30.0±0.8[b]
Week 2	28.6±0.6[a]	30.0±2.3[b]
Week 3	29.3±2.1[a]	30.0±0.6[b]
Week 4	29.4±0.9[a]	29.9±0.5[a]
Temperature, °C, 2 pm		
Week 1	37.6±0.9[a]	39.7±1.1[a]
Week 2	37.8±0.0[a]	38.7±1.0[a]
Week 3	38.2±1.0[a]	38.7±0.8[a]
Week 4	38.5±0.7[a]	38.3±0.9[a]
Temperature, °C, 6 pm		
Week 1	34.1±2.2[a]	36.4±1.9[b]
Week 2	34.9±0.8[a]	36.6±0.8[b]
Week 3	35.8±1.6[a]	36.2±0.9[b]
Week 4	35.3±0.9[a]	35.6±0.9[a]
Salinity, g/L		
week 1	104.0±9.0[a]	119.0±7.0[b]
week 2	100.0±5.0[a]	115.0±8.0[b]
week 3	101.0±2.0[a]	117.0±10.0[b]
week 4	99.0±4.0[a]	118.0±11.0[b]
Turbidity, cm		
week 1	21.0±4.0[a]	18.0±2.0[a]
week 2	20.0±3.0[a]	21.0±2.0[a]
week 3	20.0±4.0[a]	22.0±2.0[a]
week 4	18.0±4.0[a]	21.0±2.0[a]

Mean values in a row sharing the same superscript are not significantly different (p>0.05)

4.1.2 Nutrient parameters

Phosphate

During the first two months of the culture period, low values of phosphate were observed in the culture ponds; but during the third month it increased very rapidly (up to 167.09, 26.70 and 50.93 µg/L in the fertilized reservoir pond, control and fertilized culture ponds respectively) (Figure 4.4).

During the second month, creek 2 had significantly ($p<0.05$) lower phosphate level compared to creek 1, culture ponds and the reservoirs. However, during the third month R1 was shown to have significantly ($p<0.05$) higher phosphate level compared to the rest of the system. Also the fertilized culture ponds had significantly higher ($p<0.05$) phosphate level than the control culture ponds.

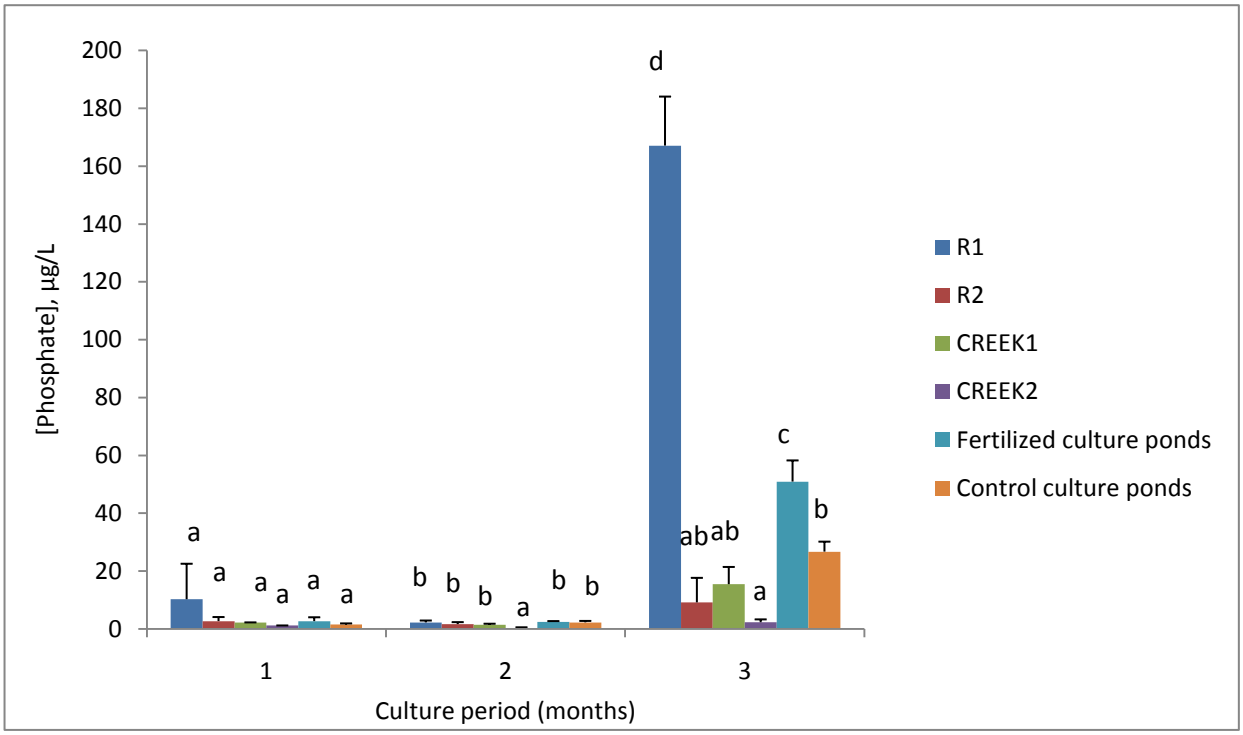


Figure. 4 Mean monthly phosphate concentrations in the water column throughout the culture system; bars are a mean of 3 replicates and error bars are the standard deviations. Means per month that do not share a superscript are significantly different at p=0.05 (one-way ANOVA); R1 stands for the fertilized reservoir pond while R2 stands for non-fertilized reservoir pond

Nitrate

Nitrate concentration was low in the first month in the entire culture system, except for creek 1 (which had a significantly higher value, $p<0.05$, than the rest of the system, except for R2), and further declined during the second month throughout the system (Figure 4.5). However, a rapid increase in nitrate concentration was observed during the third month for the entire system. In month 3, no significant differences were found throughout the system.

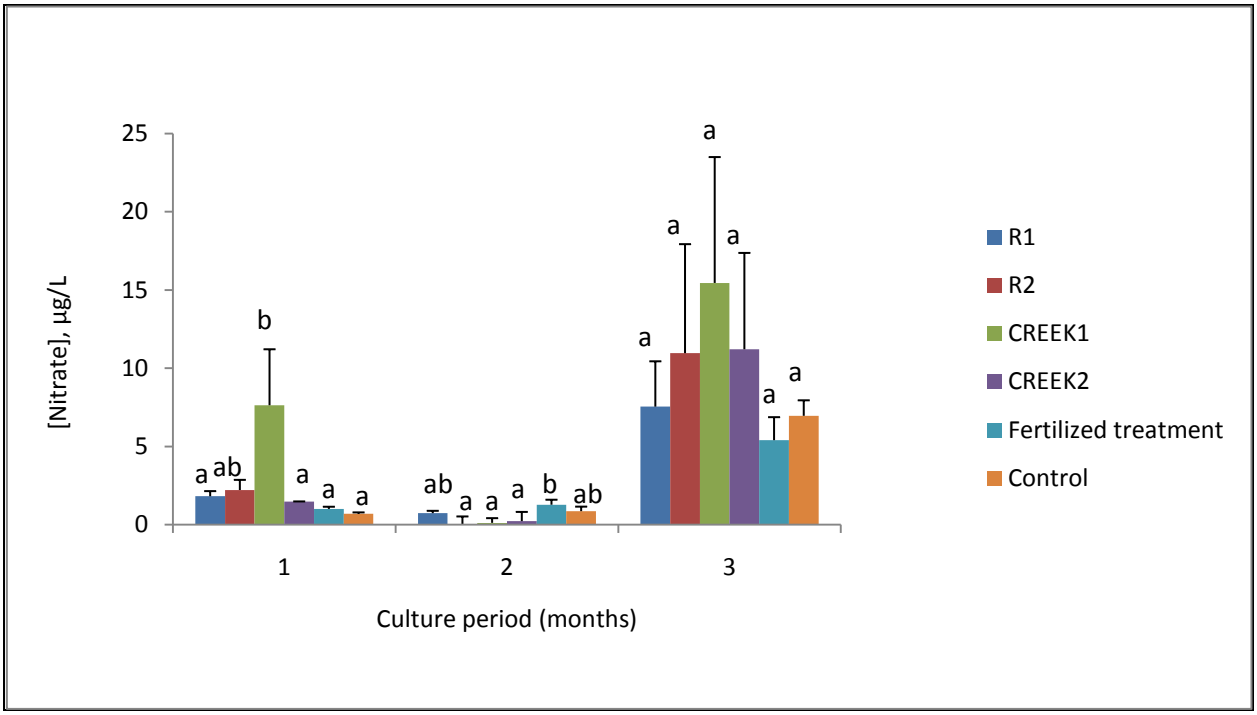


Figure 4. 5 Mean monthly nitrate concentrations in the water column throughout the culture system; bars are a mean of 3 replicates and error bars are standard deviations. Means per month that do not share a superscript are significantly different at $p<0.05$ (one-way ANOVA). R1 stands for fertilized reservoir pond while R2 stands for non-fertilized reservoir pond

Ammonia

Generally, the ammonia level dropped from the first month to the second month (Figure 4.6). A sharp increase of the ammonia levels was detected in the third month with highest levels (153.71 µg/L) observed the in the control ponds. One-way ANOVA analysis detected significantly higher ($p<0.05$) values for the control culture ponds than for both creeks and reservoirs in the third month. Also significant ($p<0.05$) difference was found in the fertilized culture ponds having higher values than both creeks and reservoir ponds in the third month.

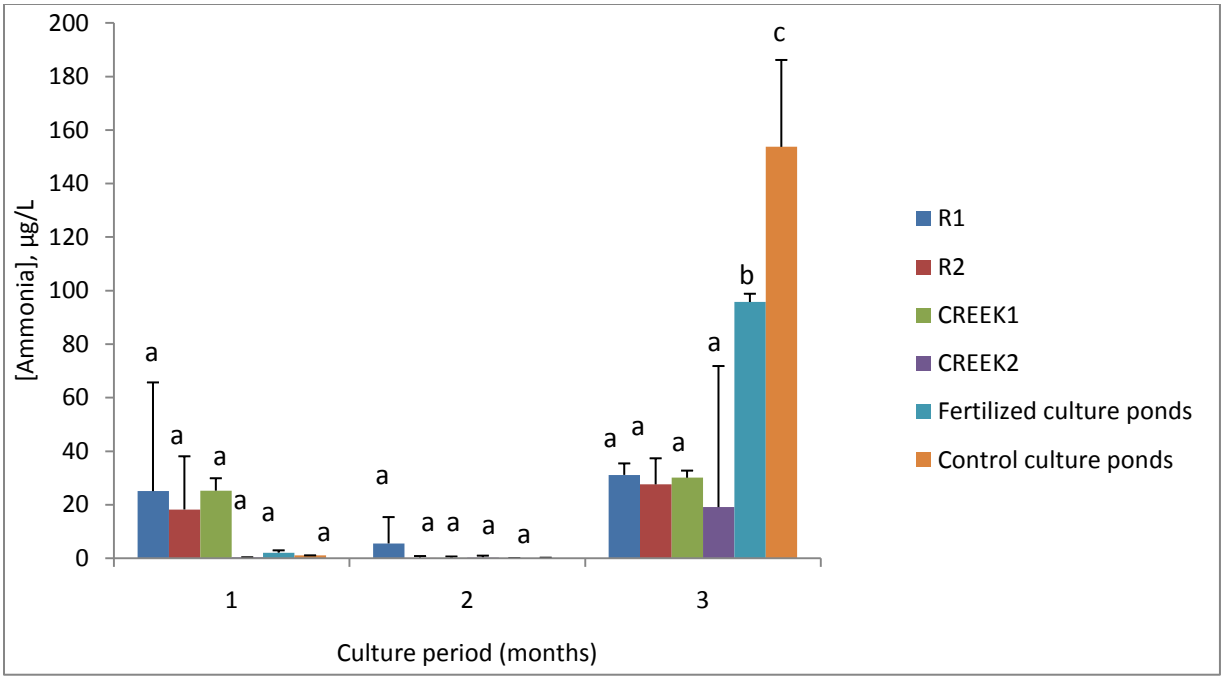


Figure 4. 6 Mean monthly ammonia concentrations in the water column throughout the culture system; bars are a mean of 3 replicates and error bars are standard deviations. Means per month that do not share a superscript are significantly different at p=0.05 (one-way ANOVA). R1 stands for the fertilized reservoir pond while R2 stands for non-fertilized reservoir pond

Effect of time and treatment on nutrient parameters

A two-way ANOVA analysis was performed to explore the interaction effect of time and treatment (Table 4.2) on nutrient parameters.

Table 4. 2 Effect of time and treatment on nutrient parameters (p-values, two-way ANOVA)

Nutrient parameter	Phosphate	Nitrate	Ammonia
Treatment	0.0006*	0.0543	<0.0001*
Time	<0.0001*	<0.0001*	<0.0001*
Interaction	<0.0001*	0.0057	<0.0001*

* designates significance at p=0.05.

Table 4. 3 Mean monthly values for the nutrient parameters (mean ± StDev. of 9 observations) in the control and fertilized culture ponds over the entire culture period

Parameter	Fertilized culture ponds	Control culture ponds
Phosphate, µg/L		
Month 1	2.67±1.33	1.51±0.38
Mont 2	2.46±0.23	2.25±0.49
Month 3	50.93±7.31*	26.70±3.45*
Nitrates, µg/L		
Month 1	1.00±0.14	0.70±0.08
Month 2	1.27±0.32	0.86±0.29
Month 3	5.40±1.47	6.96±0.99
Ammonia, µg/L		
Month 1	2.10±0.88	1.06±0.05
Month 2	0.19±0.10	0.18±0.23
Month 3	95.74±3.09*	153.71±32.46*

Means of a given parameter and month that have an asterisk are significantly different at p= 0.05 (two-way ANOVA)

Interaction effect of time and fertilized treatment on phosphate, nitrate and ammonia is shown in Table 4.2 and 4.3. There was significantly ($p<0.05$) higher phosphate level in the fertilized culture ponds during the third month. For nitrate no significant difference was shown throughout the culture period while for ammonia, significantly higher ($p<0.05$) values were shown in the control and the fertilized culture ponds in the third month (Table 4.2 and 4.3). Generally lower levels of phosphate, nitrate and ammonia were observed in all ponds during the first and second month of the culture period.

4.1.3 Phytoplankton and non-*Artemia* zooplankton found in the experimental ponds

Different phytoplankton and zooplankton species were observed in control ponds, fertilized culture ponds, creeks, and reservoir ponds, although sometimes some species were also observed in more than one system (Table 4.4). Some of the organisms observed acted as predators to *Artemia* and some acted as competitors for food.

Phytoplankton

Harmful algae that produce toxins and cyanotoxins, diatoms and other phytoplankton groups were observed in the ponds and creeks. Although fluctuation in species dominance was observed at different times, some species were showing up throughout the experimental culture periods. In creek 1, *Pleurosigma sp.* dominated the species population in month 1 and 2 (10,775 and 1,250 cells/L, respectively) while toxic algae (*Oscillatoria sp.*) ranked top in the population during the last month (Table 4.4). In creek 2, *Peurosigma sp*. (3,275 cells/L), *Pleurosigma sp.* (2,325 cells/L) and *Anabaena sp.* dominated during the first, second and third months consecutively. In the fertilized reservoir pond, *Oscillatoria sp.* (375 cells/L), *Pleurosigma*

sp. (3,875 cells/L) and *Anabaena sp.* (700 cells/L) dominated the species population in the first, second and third months consecutively.

On the other hand, *Pleurosigma sp.* (4,625 cells/L), *Nitzschia sp.* (66 cells/L) and *Anabaena sp. (*1,525 cells/L) dominated the species composition during the first, second and third months respectively in the non-fertilized reservoir pond. In the control culture ponds, a high concentration of *Oscillatoria sp.* (8,083 cells/L) was observed in the first month, followed by *Pramyclamis vectnesis* and *Pramyclamis sp*. (122 cells/L) in the second month and *Anabaena sp.* (4,925 cells/L) in the third month. Lastly, in the fertilized culture ponds, *Oscillatoria sp.* (1,742 cells/L), *Pramyclamis sp.* (111 cells/L) and *Anabaena sp.* (942 cells/L) dominated the population during the first, second and third months respectively.

Highest total abundance of phytoplankton cells was recorded in the non-fertilized reservoir pond (average number of 33,525 cells/L) during the first month, followed by creek 1 (average number of 19,150 cells/L) in the first month. The lowest algal concentration was recorded in the fertilized culture ponds (average number of 1,408 cells/L) in the third month (Table 4.4).

Table 4. 4 Phytoplankton species diversity and abundance in the culture ponds, creeks and reservoir ponds; abundance unit = total number of cells/L

System/Pond	Month	Phytoplankton species and abundance	Total phytoplankton abundance
Creek 1	1	*Pleurosigma sp.* (10,775), *Novicula sp.* (3,775), *Ostreopsis sp.**(125), *Protoperidinium sp.* (500), *Fragilaria sp.* (75), *Nitzschia closterium** (1,725), *Oscillatoria sp.***(1,300), *Scrippsiella sp.****(150), *Coscinodiscus sp.****(25), *Pechastrum sp.* (25), *Anabaena sp.* (25), *Pramyclamis vectnesis* (650)	19,150
	2	*Thalassionema nitzschioides* (25), *Anabaena sp.* (225), *Pleurosigma sp.* (1,250), *Pseudo-nitzschia sp.* (50), *Oscillatoria sp.*** (525), *Nitzschia closterium** (225), *Chaetocerous sp.* (25), *Protoperidinium sp.* (175), *Scrippsiella sp.**** (125), *Navicula sp.* (225), *Microcystis colony sp.* (75), *Chaetocerous sp.* (50), *Nitzschia sp.* (25), *Coscinodiscus sp.**** (25), *Prorocentrum sp.** (25), *Spirulina sp.* (25)	3,075
	3	*Oscillatoria sp.*** (1,875), *Pleurosigma sp.* (50), *Navicula sp.* (75), *Anabaena sp.* (775), *Pseudo-nitzschia sp.* (125), *Dictyota sp.* (50), *Trichodesmium sp.* (25)	3,000
Creek 2	1	*Pleurosigma sp.* (3,275), *Microsystis colony sp.* (525), *Navicula sp*. (3075), *Nitzschia closterium** (625), *Peridinium sp.**** (100), *Fragilaria sp.* (25), *Oscillatoria sp.****(250), *Scrippsiella trochioidea****(25), *Coscinodiscus sp.* (50), *Pramyclamis vectnesis* (825)	9,475
	2	*Pleurosigma sp.* (2,325), *Thalassionema sp.* (25), *Oscillatoria sp.*** (150), *Microcystis colony sp.* (400), *Navicula sp.* (575), *Protoperidinium sp.* (50), *Anabaena sp.* (50), *Nitzschia sp.* (25), *Hemidiscus sp.* (25), *Pyramimonas sp*. (25), *Coscinodiscus sp.**** (75), *Scrippsiella sp.**** (25), *Dictyota* sp. (25), *Nitzschia closterium** (75)	3,850
	3	*Navicula sp.* (125), *Oscillatoria sp.*** (650), *Anabaena sp.* (3,725), *Pleurosigma sp.* (75), *Scenedesmus sp.* (300), *Pechastrum sp.* (175), *Oestropsis sp.* (25), *Pseudo-nitzschia sp.* (25), *Protoperidinium sp.* (25), *Trichodesmium sp.* (50), *Synura sp*. (25)	5,200

Table continued

Fertilized reservoir pond	1	*Oscillatoria sp.* (375), *Pleurosigma sp.* (350), *Nitzschia closterium** (175), *Navicula sp.* (225), *Pramyclamis vectnesis* (300)	1,425
	2	*Pleurosigma sp.* (3,875), *Oscillatoria sp.*** (450), *Navicula sp.* (50), *Protoperidinium sp* (75), *Fragilaria sp.* (50)	4,500
	3	*Anabaena sp.* (700), *Pechastrum sp.* (25), *Pramyclamis* sp. (575), *Pleurosigma sp.* (25), *Oscillatoria sp.* (175), *Dictyota sp.* (75), *Coscinodesmus sp.* (25)	1,600
Non-fertilized reservoir pond	1	*Pleurosigma sp.* (4,625), *Navicula sp.* (1,800), *Ostreopsis sp.** (100), *Fragilaria sp.* (50), *Nitzschia closterium* *(2,3075), *Oscillatoria sp.* (2425), *Choanoflagellida sp.* (75), *Coscinodiscus sp.**** (25), *Coccolith sp.* (25), *Anabaena filament* (50), *Pramyclamis vectnesis* (1,250)	33,525
	2	*Pleurosigma sp*. (52), *Pramyclamis sp.* (7), *Oscillatoria sp.*** (5), *Coscinodiscus sp.****(2), *Nitzschia sp.* (66), *Protoperidinium sp.* (60), *Navicula sp.* (5), *Spirulina sp.* (1), *Microcystis colony sp.* (1), *Nitzschia closterium** (3), *Anabaena sp.* (2)	5,100
	3	*Anabaena sp.* (1,525), *Pechastrum sp.* (25), *Pramyclamis sp.* (25), *Pleurosigma sp* (300), *Oscillatoria sp.* (675), *Scenedesmus sp* (25)	2,575
Control culture ponds	1	*Navicula sp* (167) *Oscillatoria sp.* (8,083), *Pleurosigma sp* (108), *Nitzschia closterium** (92), *Anabaena sp.* (75), *Pramyclamis vectnesis* (2,700), *Protoperidinium sp.* (1,225), *Lyngbya sp.* (25), *Anabaena filament* (188), *Pramyclamis sp.* (100), *Pyramimonas sp.* (225)	11,842
	2	*Nitzschia closterium** (14), *Pramyclamis vectnesis* (122), *Dunaliella sp.* (1), *Naviculla sp*. (9), *Pleurosigma sp.* (11), *Pramyclamis sp.* (122)	3,908
	3	*Pramyclamis sp.* (38), *Oscillatoria sp.*** (633), *Anabaena sp.* (4,925), *Naviculla sp.* (108), *Pechastrum sp.* (375), *Pleurosigma sp.* (88), *Dictyota sp.* (25), *Cocolith sp.* (100), *Scenedesmus sp.* (50), *Dictyota sp.* (25), *Cocolith sp.* (100)	2,075
Fertilized culture ponds	1	*Pramyclamis sp.* (525), *Protoperidinium sp.* (888), *Nitzschia closterium**(363), *Navicula sp.* (208), *Pleurosigma sp.* (400), *Pyramimonas sp.* (100), *Oscillatoria sp.*** (1,742), *Anabaena sp.* (50), *Pramyclamis sp.* (1,075), *Protoperidinium sp.* (25),	4,100
	2	*Prymnescum sp.* (2), *Pramyclamis sp.* (111), *Oscilatoria sp.*** (2), *Dunaliella sp.* (3), *Nitzschia sp.* (1), *Pleurosigma sp.* (1), *Navicula sp.* (2), *Nitzschia closterium** (2)	2,908
	3	*Pramyclamis sp.* (163), *Anabaena sp*. (942), *Oscillatoria sp.***(183), *Navicula sp.* (50), *Pechastrum sp.* (25), *Scenedesmus sp.* (25), *Pleurosigma sp.* (25), *Dictyota sp.* (25), *Pediastrum sp.* (125), *Nitzschia sp.* (50), *Dictyota octonaiva* (25)	1,408

N.B: Number in brackets refers to the average number of phytoplankton cells/L. For control and fertilized culture ponds, the number in brackets is the mean of 3 replicates. A single * denotes toxic algae, double * denotes cyanotoxic while triple * stands for toxic as blooms

Zooplankton

Acartia sp. (118 and 207 individuals/L) dominated the zooplankton in the control and the fertilized culture ponds respectively during the first month. Also, *Harpacticoid sp.* (137 individuals/L) dominated the zooplankton in the control culture ponds during the second month. Other species observed were fewer than 100 individuals/L (Table 4.5) throughout the culture period. Generally, *Harpacticoid* copepods, polychaetes, *Brachionus sp.,* medusa and mollusks larvae were observed in the creeks and experimental ponds.

Table 4. 5 Zooplankton species and densities observed in the creeks, culture ponds and reservoir ponds

	Month	Taxa (Species and individuals/ L)
Creek 1	1	*Carnuelaa perplexa* (56), *Turbellaria sp.* (1)
	2	*Ostracod sp.* (2), *Acartia sp.* (31), *Harpacticoid sp.* (8), *Medusa* (2), *Turbellaria sp.* (2), *Thermosbaenacea sp.* (9), *Undinula s.p* (2)
	3	*Acartia negligens* (317), *Harpacticoid sp.* (7), *Ostracoda sp.* (3), Insect sp. (1), *Turbellaria sp.* (19), *Undinula sp*. (10), *Temora sp.* (1), Nematoda sp. (1), *Centropages sp.* (1)
Creek 2	1	*Culicoides larva* (2), *Carnuelaa perplexa* (1), *Acartia sp.* (17), *Undinula sp.* (14), Fish eggs (1), *Polychaeta sp.* (1)
	2	*Culicoides* larvae (2), *Harpacticoid sp.* (10), *Undinula sp.* (9), *Medusa* (14), *Ostracod sp.* (3), *Oithona* (1), *Brachyura sp.* (3)
	3	*Acartia sp.* (16), Mysids (15), *Undinula sp.* (11)
Fertilized reservoir pond	1	*Turbellaria sp.* (1), *Acartia sp.* (81), *Undinula sp.* (46), *Harpacticoid sp.* (13), *Temora sp.* (1)
	2	*Harpacticoid sp.* (15), *Polychaeta sp.* (15), *Undinula sp.* (3), *Acartia sp.* (2), Insect (1), *Turbellaria sp.* (17)
	3	*Acartia sp.* (6), *Harpacticoid sp.* (3), *Undinula sp.* (2)
Non-fertilized reservoir pond	1	*Acartia sp*. (3), *Undinula sp.* (52), *Harpacticoid sp.* (12)
	2	*Turbellaria sp.* (8), *Harpacticoid sp.* (15), *Acartia sp.* (12), *Labidocera sp.* (1), *Culicoides* larvae (1), *Undinula sp.* (1), *Oithona sp*. (2)
	3	*Acartia sp.* (58), *Undinula sp.* (89), *Turbellaria sp.* (11), *Harpacticoid sp.* (19), Nematoda (2), Bivalve (1)
Control culture ponds	1	*Carnuelaa perplexa* (16), *Turbellaria sp.* (4), *Undinula sp.* (1), *Acartia sp.* (118), *Oithona sp.* (1), *Oncaea sp.* (1), *Harpacticoid sp.* (26)
	2	*Harpacticoid sp.*(137), *Turbellaria sp.* (30), *Culicoides* larvae (8), *Thermosbaenacea sp.* (1), *Undinula sp.* (1), *Temora sp*. (1), Insect *sp.* (1),
	3	Insect sp. (1), *Harpacticoid sp.* (5), *Rotifer* sp. (18)
Fertilized treatment Culture ponds	1	*Harpacticoid sp.* (6), *Undinula sp.* (38), *Acartia sp.* (207)
	2	*Harpacticoid sp.* (34), *Turbellaria sp.* (15), *Thermosbaenacea sp.* (48), *Culicoides* larvae (5)
	3	*Harpacticoid sp.* (21), Insect sp. (1), *Rotifer sp.* (12)

N.B: Number in brackets refers to the average number of zooplankton individuals/L. For control and fertilized culture ponds, the number in brackets is the mean of 3 replicates.

4.1.4 *Artemia* growth and production parameters

4.1.4.1 Population density, composition and growth

Population density

Population density showed a slight decrease at day 14 as compared to the first weeks. However, a rapid increase in population density was then observed from day 21 onwards, reaching a maximal average value (950 animals/L) at day 28 in the fertilized culture ponds (Figure 4.7). The fertilized culture ponds showed significantly ($p=0.0048$ and $p=0.0111$) higher densities in the last 2 weeks of the culture than the control culture ponds.

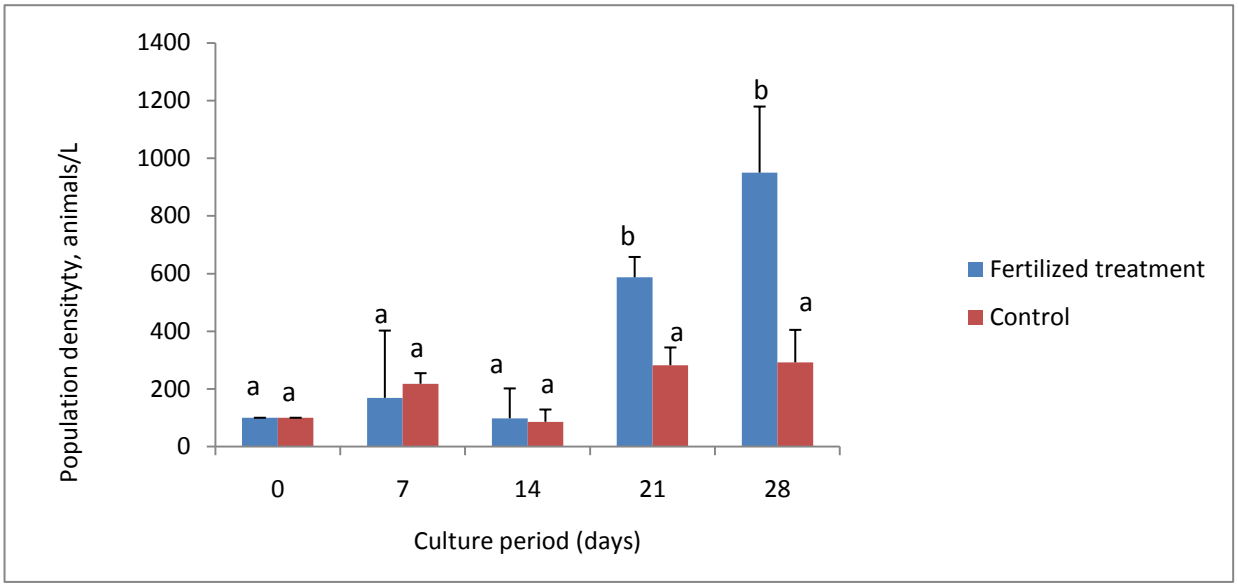
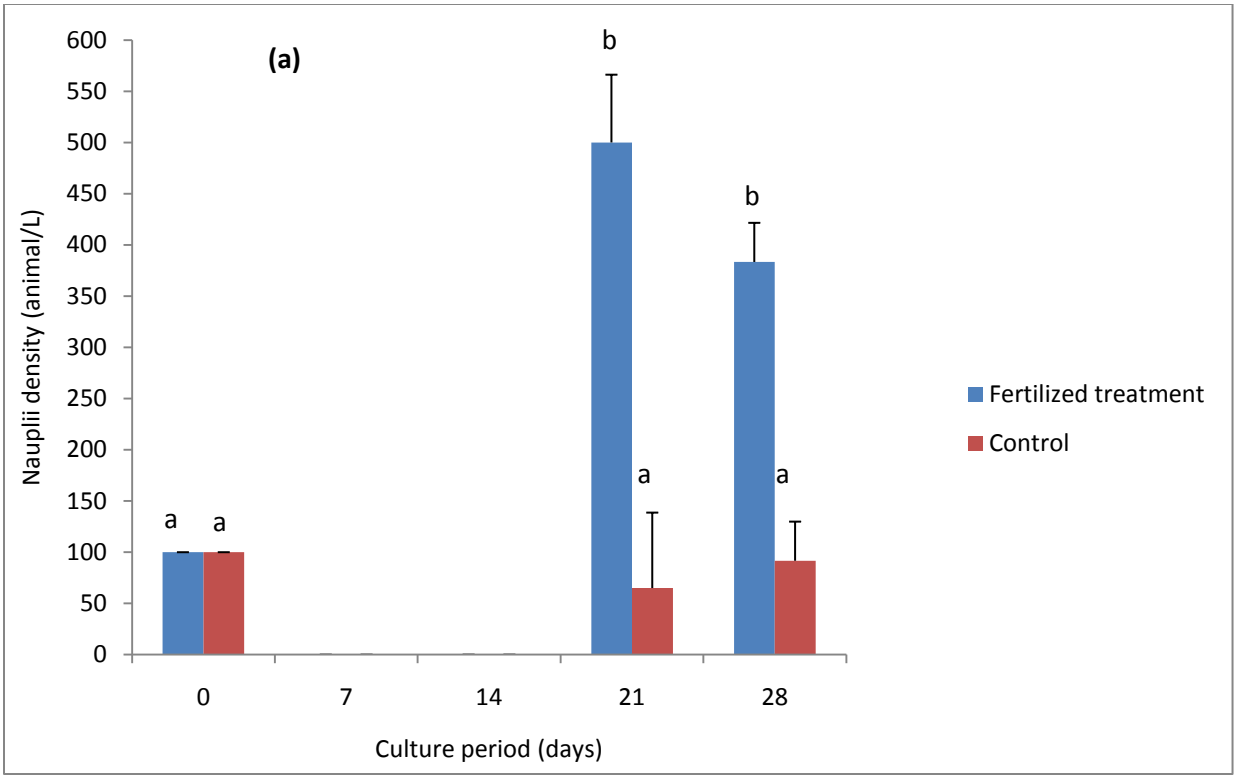


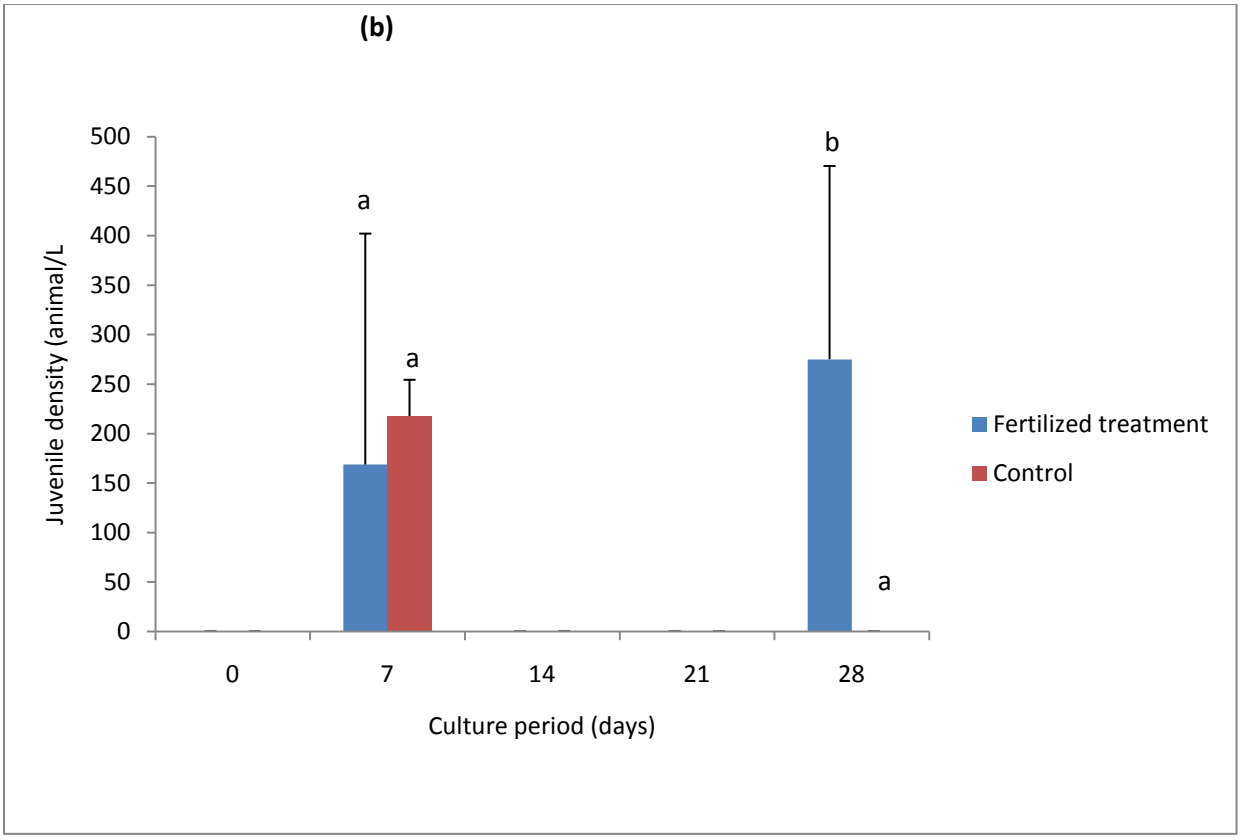
Figure 4. 7 Mean weekly population density; bars are means of 3 replicates and error bars are standard deviations. Means per week sharing the same superscript are not significantly different ($p>0.05$).

Population composition

Figures 4.8a, b and c summarize the population composition data (i.e. nauplii, juveniles and adults density respectively). Through the density of the respective age classes the growth and maturation of the population of the inoculated *Artemia* nauplii can be followed. There were no nauplii observed at day 7 and 14 indicating the growth of nauplii into juvenile and adult stages. As from day 14, the adult population was first observed when juveniles grew into the adult stage. Nauplii were observed again after day 21 when adults started to release nauplii. The highest naupliar density (500 nauplii/L) was recorded at day 21 in the fertilized culture ponds (Figure 4.8a).

Apart from day 7, juveniles were only observed at the very end and only in the fertilized culture ponds (275 individuals/L) (Figure 4.8b). The fertilized culture ponds showed significantly ($p=0.0074$) higher nauplii density after day 21 and 28. Also significantly ($p<0.05$) higher juvenile densities were recorded after day 28. There was never any significant difference in adult densities between the control and the fertilized culture ponds.


(a)
Nauplii density (animal/L)
600
550
500
450
400
350
300
250
200
150
100
50
0
a
a
b
a
b
a
0
7
14
21
28
Culture period (days)
Fertilized treatment
Control


(b)
Juvenile density (animal/L
500
450
400
350
300
250
200
150
100
50
0
a
a
b
a
0
7
14
21
28
Culture period (days)
Fertilized treatment
Control

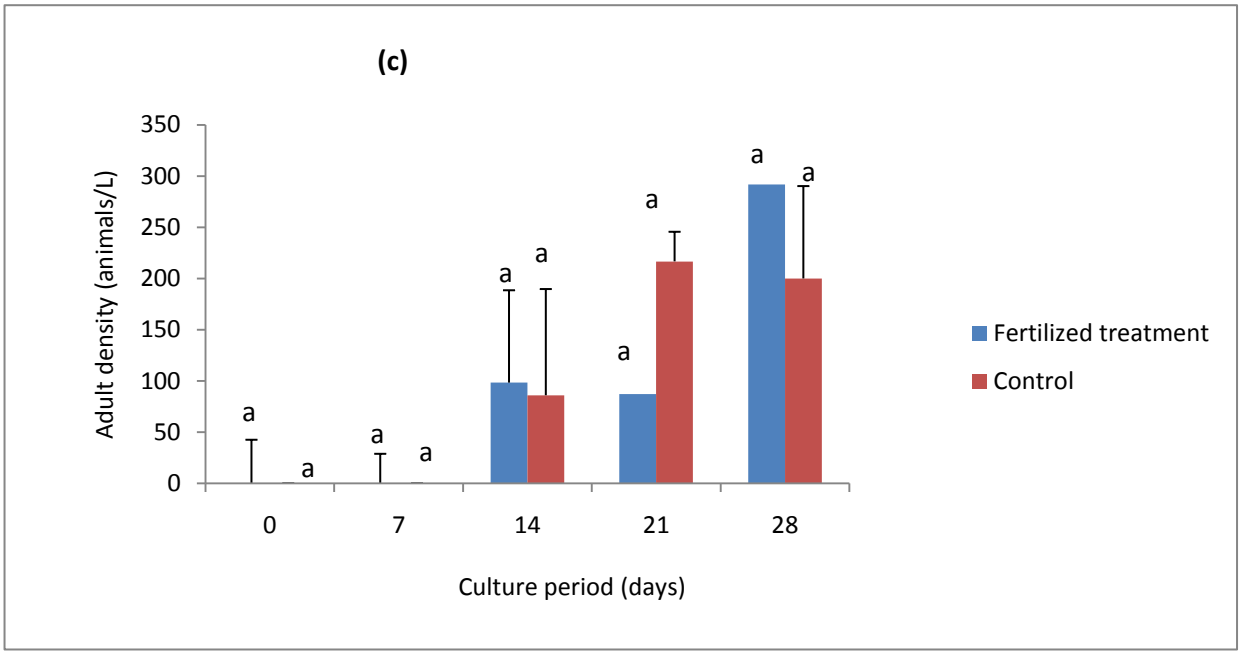


Figure 4. 8a,b and c Mean weekly density of various age classes; a: nauplii, b: juveniles and c: adults, points are a mean of 3 replicates. Mean bars per week sharing the same superscript are not significantly different ($p>0.05$).

Maturity

At day 14, maturation percentage observed in the fertilized culture ponds was 69.2%±7.1, while it was 30.3%±13.3 in the control. There was no significant difference between both values ($p=0.2157$).

Dry weight (DW)

Dry weight increased from day 7 to day 28 (Figure 4.9). The animals in the fertilized culture ponds were bigger than those in the control. The significant ($p<0.05$) differences found were in fertilized culture ponds having higher dry weight than the control culture ponds at days 14 and 21 of the culture period.

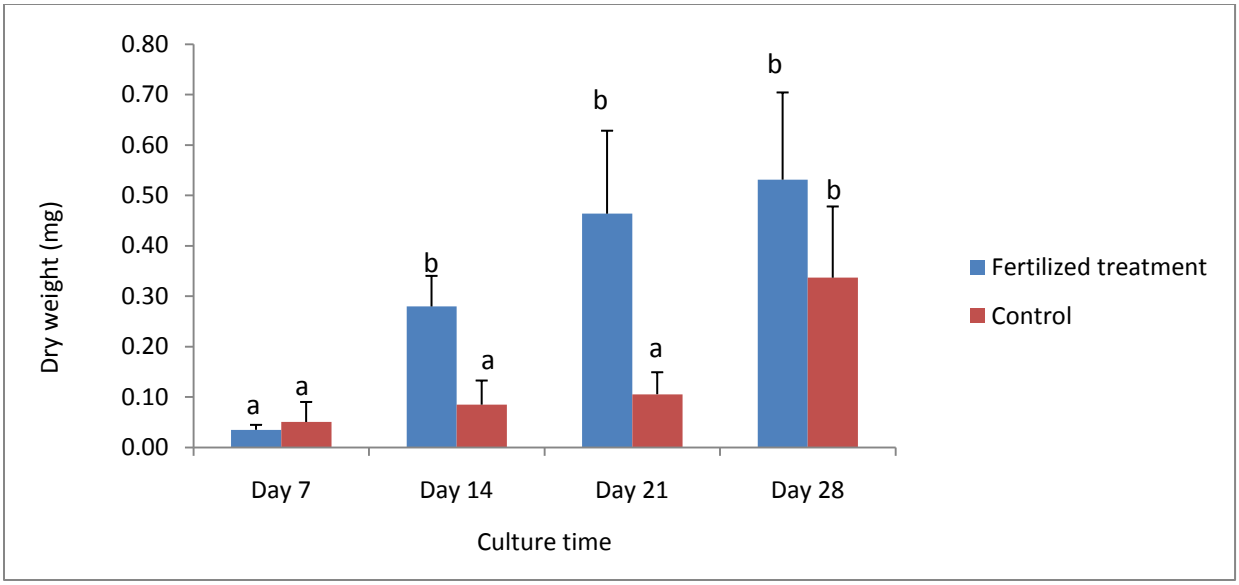


Figure 4. 9 Mean weekly dry weight; error bars are standard deviations; bars are a mean of 3 replicates over the entire culture period. Mean bars per week sharing the same superscripts are not significantly different (p>0.05).

Total length

The animals increased in length from day 7 to day 28; growth was fastest for the animals in the fertilized culture ponds (Figure 4.10), and the difference was significant (p<0.05) from day 14 onwards, resulting in a final value of 7.2 cm for the fertilized culture versus 4.8 cm for the control culture ponds.

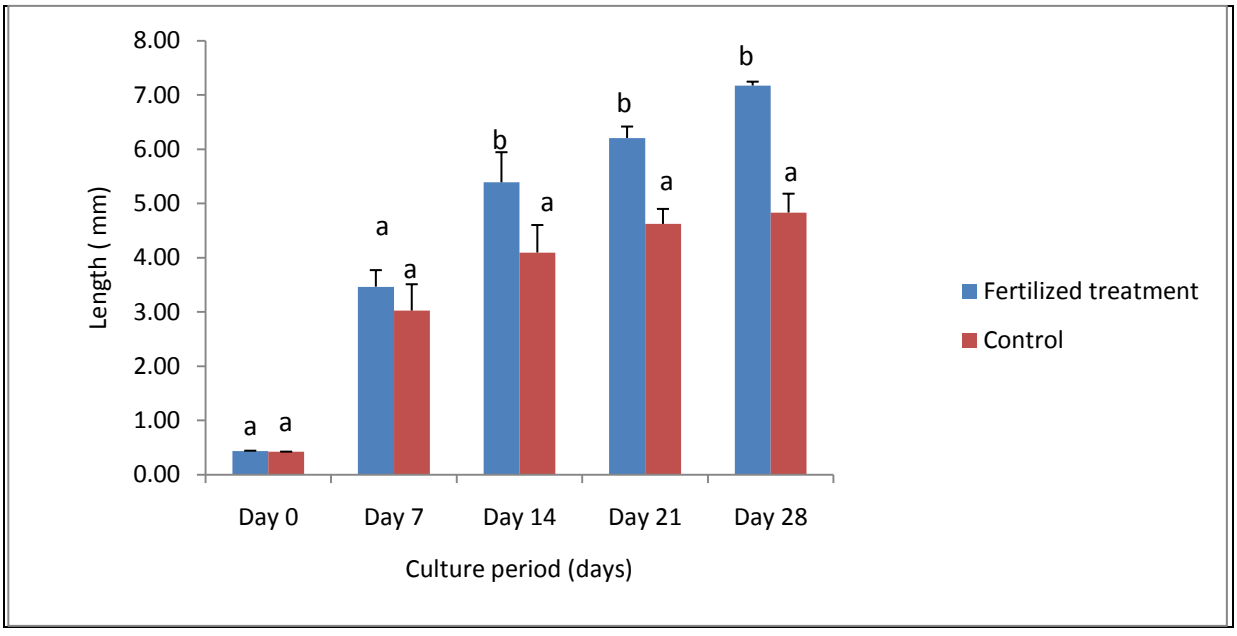


Figure 4. 10 Mean weekly total length; error bars are standard deviations; bars are a mean of 3 replicates per week. Mean bars per week sharing the same superscripts are not significantly different (p>0.05).

Length- weight relationship

Figures 4.11a and b show the existence of a moderate length-weight correlation (correlation coefficient 0.8660-0.7047).

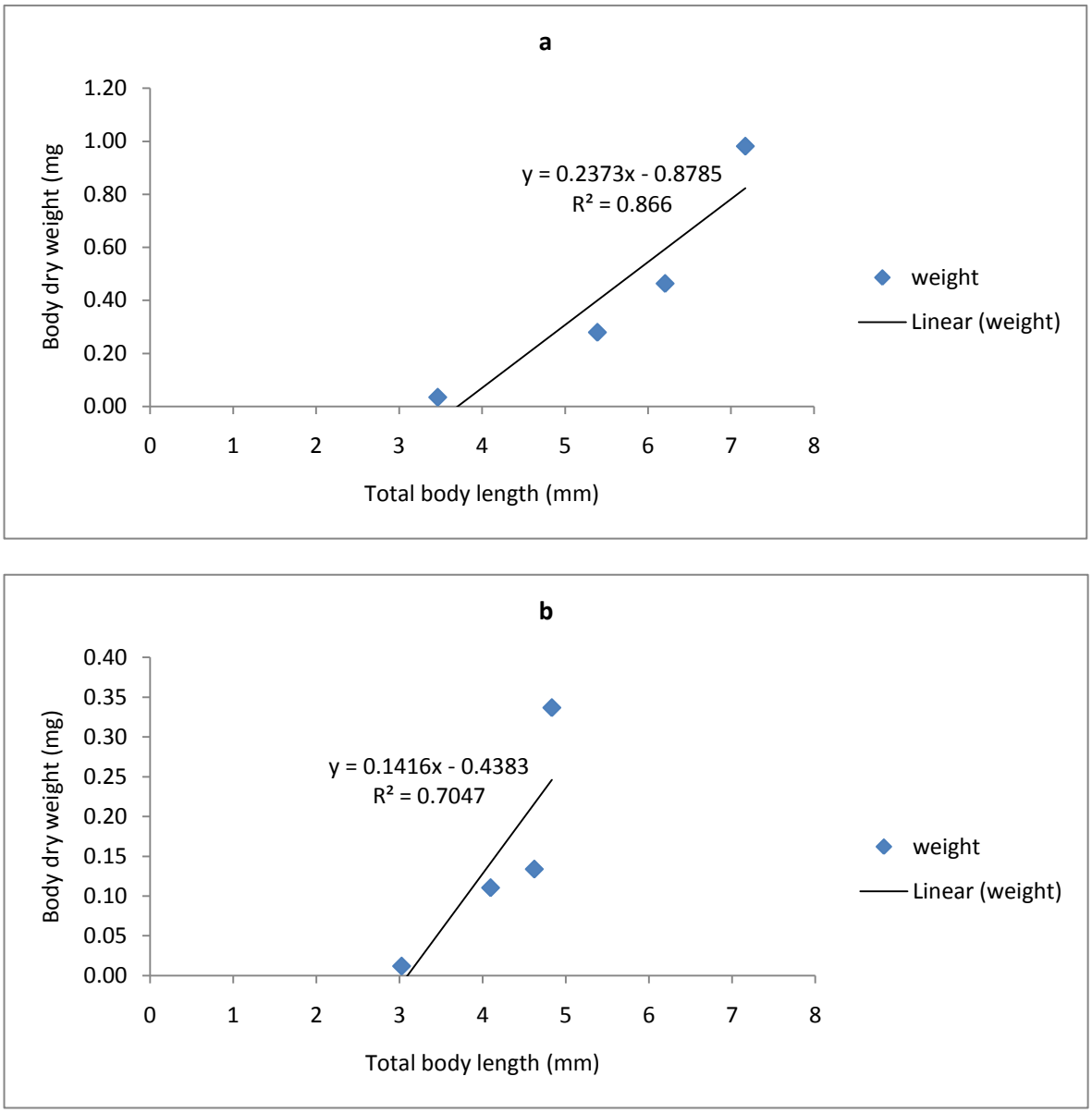


Figure 4. 11a and b Length/weight relationship *Artemia* in culture trial: a) fertilized culture ponds; b) control culture ponds

4.1.4.2 *Artemia* reproduction parameters

Percentage oviparity

Average percentage oviparity reached a peak (69%) in week 4 for the fertilized culture ponds while for the control it reached a peak (44%) in weeks 3 and 4 (Table 4.7). Generally, oviparity was rapidly increasing from weeks 2 to 4 (4 – 69%) for the fertilized treatment while in the control it rose from 20 to 44% in weeks 2 – 3 and remained stable in week 4 (Figure 4.12).

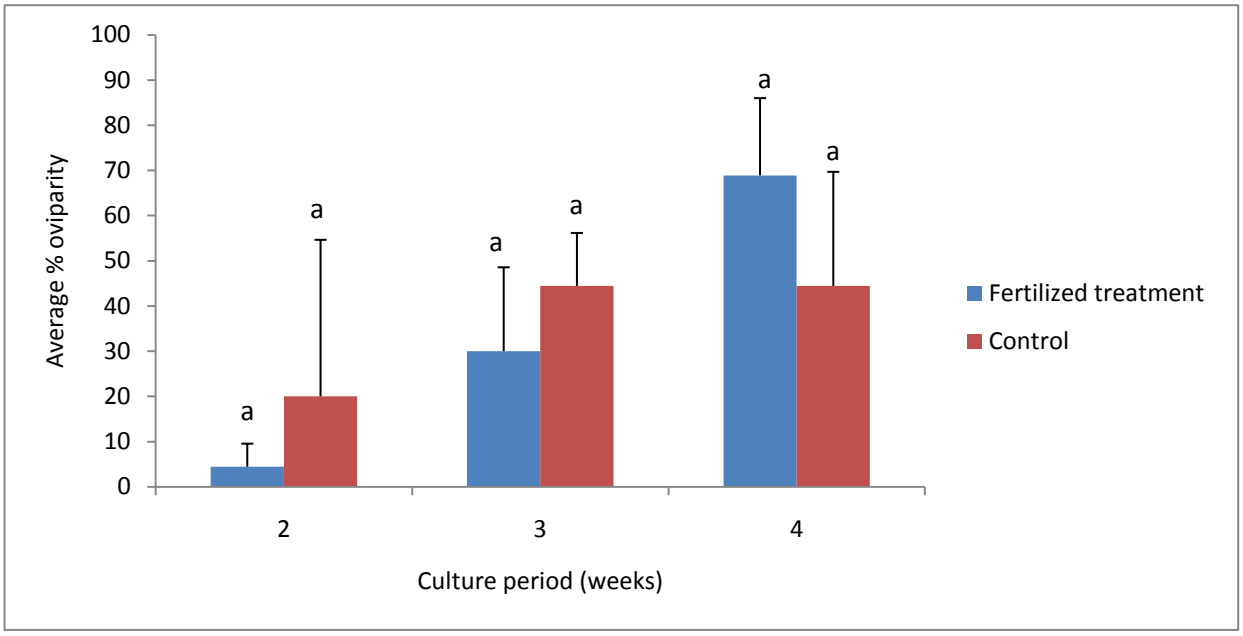


Figure 4. 12 Mean weekly oviparity (%); error bars are standard deviations; bars are a mean of 3 replicates. Mean bars per week sharing the same superscript do not differ significantly ($p>0.05$)

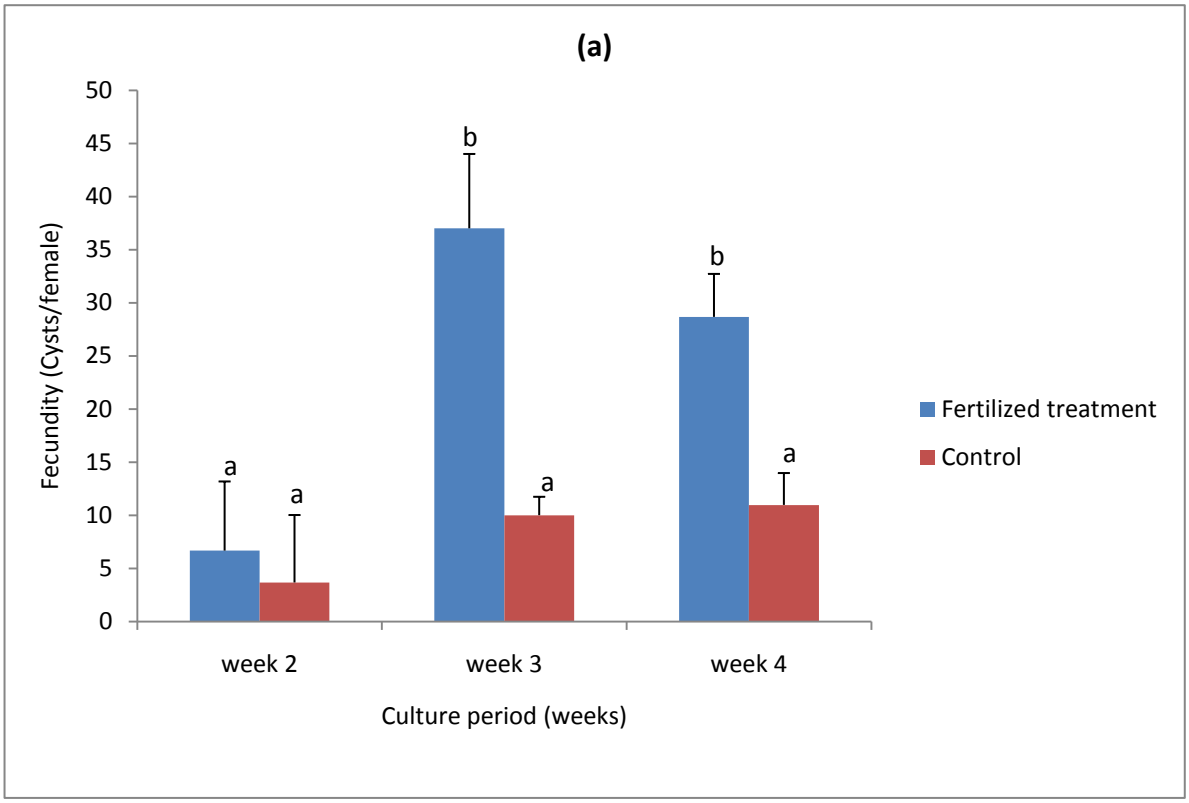
There was no significant ($p>0.05$) difference in percentage oviparity between the control and the fertilized culture ponds for the entire culture period.

Fecundity of oviparous females

Generally, the highest oviparous fecundity was observed in the fertilized culture ponds with 37 cysts per female in week 3, while the lowest fecundity was found in the control culture ponds (4 cysts) in week 2 (Table 4.7). A decline in fecundity occurred in week 4 for the fertilized culture ponds but there was a gradual though small increase in oviparous fecundity for the control throughout the culture period (Figure 4.13a). In weeks 3 and 4, the fertilized culture ponds showed significantly ($p<0.05$) higher brood size than the control in those weeks, and than the values for both fertilized and control culture ponds in week 2.

Fecundity of ovoviviparous females

Like in oviparity, highest fecundity was observed in the fertilized culture ponds with a maximal value of 40 nauplii per female in week 3 (Table 4.7). A turn down of fecundity occurred in week 4 for the fertilized culture ponds while a regular but small increase in fecundity was observed for the control (Figure 4.13b). Significantly ($p<0.05$) higher brood size was shown in the fertilized culture ponds than in the control in weeks 2 and 3.



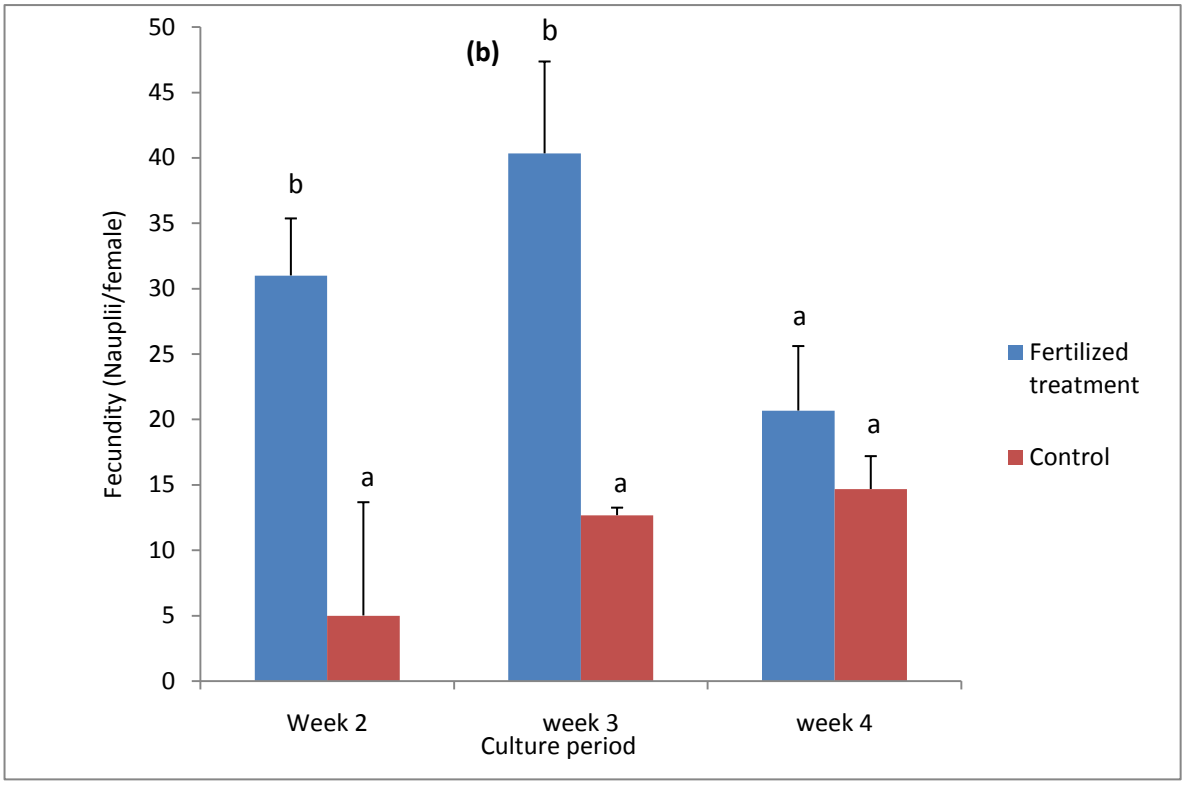


Figure 4. 13a and b Mean weekly fecundity (a: cysts/female and b: nauplii/female), error bars are standard deviations, while bars are a mean of 3 replicates. Mean bars per week sharing the same superscript do not differ significantly ($p>0.05$).

Artemia cysts yield

Cyst yield decreased from week 4 to the end of the culture period in the fertilized culture ponds, while it was minimal in the control ponds throughout the culture period, with some yield observed only in week 5 and 6 (Table 4.7). In general, mean weekly cyst yield for the fertilized culture ponds was highest in week 4 (61.10 gDW, Figure 4.14). The lowest amount observed was 1.53 gDW in the control culture ponds. In week 4 and 5, fertilized culture ponds showed significantly ($p<0.05$) higher cyst yield compared to the control culture ponds.

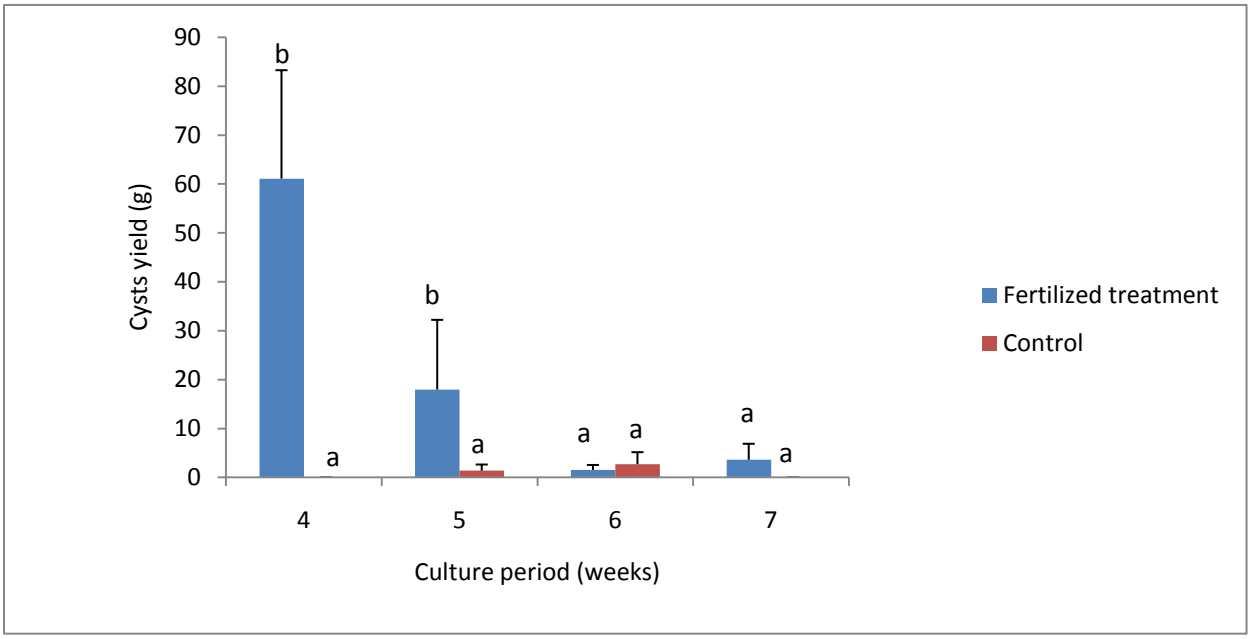


Figure 4. 14 Mean weekly cyst yield from week 4 to 7 of the culture period; error bars are standard deviations, bars are averages of 3 replicates. Mean bars per week sharing the same superscript do not differ significantly ($p>0.05$).

The combined effect of time and treatment on *Artemia* population density, growth and reproduction

The two way ANOVA analysis for interaction effect of time and fertilization treatment on *Artemia* parameters (Table 4.6) revealed significant ($p<0.05$) interaction between time and fertilization treatment for all parameters analyzed.

Table 4. 6 Effect of time and treatment on production parameters (p-values, two-way ANOVA)

Parameter	Cyst yield	Population density	Oviparous fecundity	Ovoviviparous fecundity	DW	Length
Treatment	0.0014*	0.0199*	0.0036*	0.0004*	0.0402*	0.0053*
Time	0.0688	<0.0001*	0.0005*	0.0572	<0.0001*	<0.0001*
Interaction	0.0358*	0.0003*	0.0093*	0.0250*	0.0171*	<0.0001*

Population density (individuals/ L), total length (mm), dry weight (mg), cyst yield (g), fecundity (cysts/female, nauplii/female), an ***** indicates a significance at p=0.05

Table 4. 7 Mean weekly cyst yield, fecundity estimates and percentage oviparity (mean ± StDev. of 3 observations) of the control and the fertilized culture ponds over the entire culture period, a row with an asterisk indicates a significant difference at p=0.05

Treatment	Fertilized culture ponds	Control culture ponds
Oviparious fecundity, cysts/female		
week 2	7.00±7.00	4.00±6.00
week 3	37.00±7.00*	10.00±2.00*
week 4	29.00±4.00*	11.00±3.00*
Ovoviviparous fecundity, nauplii/female		
week 2	31.00±4.00*	5.00±9.00*
week 3	40.00±7.00*	13.00±1.00*
week 4	21.00±5.00	15.00±3.00
% Oviparity		
week 2	4.44±5.09	20.00±34.64
week 3	30.00±18.56	44.44±11.71
week 4	68.89±17.10	44.44±25.24
Cyst yield		
week 4	61.10±22.17*	0.00±0.00*
week 5	17.97±14.25*	1.37±1.26*
week 6	1.53±1.01	2.71±2.45
week 7	3.60±3.28	0.00±0.00

4.2 Socio-economic study

Out of 41 villagers questioned, 56% were males while 44% were females (Figure 4.15). Some of those individuals questioned were working at the project site, some were used to work there during the time of constructing the ponds, some were indirect beneficiaries of the project as their relatives or families were working or used to work at the project farm and some were the workers at the Kensalt farm.

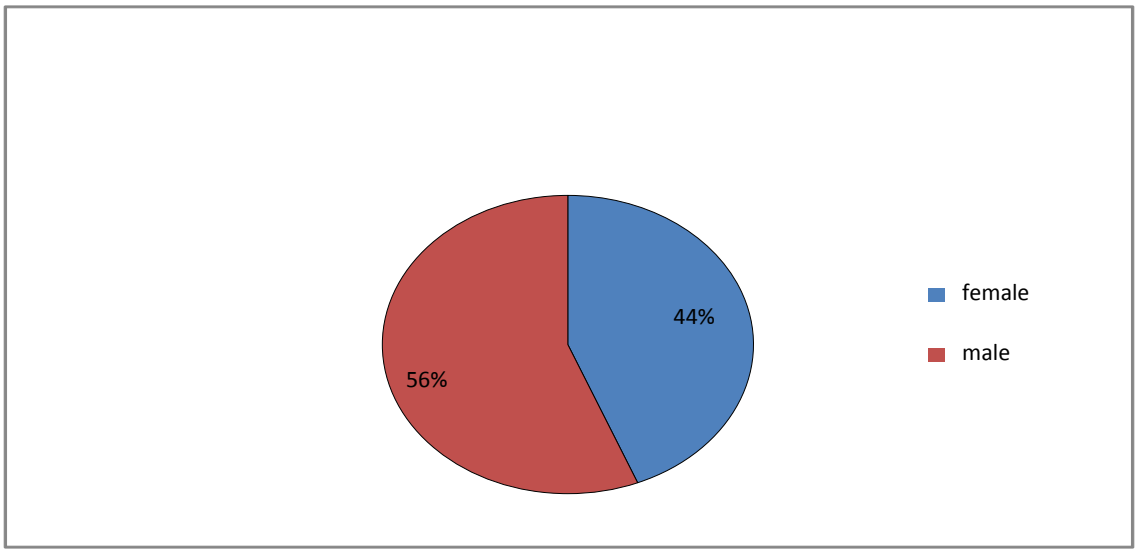


Figure 4. 15 Sex distribution of the villagers who were interviewed concerning the *Artemia* project at Kadzuhoni

The mean age of the respondents was 39 yrs with a standard deviation of 16. The oldest person who responded to the questionnaires was 80 yrs while the youngest was 18 yrs old. The average house hold size was 8 people with average total expenditure of 323.5 Kshs, which is equivalent to 3.24 € per day.

Project awareness

About 95 % of the villagers were aware of the ongoing project of *Artemia* culture at Kadzuhoni. Very few people did not have any idea on the *Artemia* culture and its use in general.

Appreciation of the project valuability

About three quarters of the interviewees showed their appreciation to the *Artemia* culture project. Most of those villagers who thought of the project as being valuable to them were the direct and indirect beneficiaries i.e. they or their relatives participated by selling their labour to the actual construction of the *Artemia* ponds or were still employed at the farm.

Children's occupation

A high number of children were still schooling followed by those who had various activities like fishing, casually working, farming or making and selling local palm beer. These were the children of the household, and the head of the house specified the occupation of his/her children. Very few children of the house hold were employed at Kensalt farm (Figure 4.16).

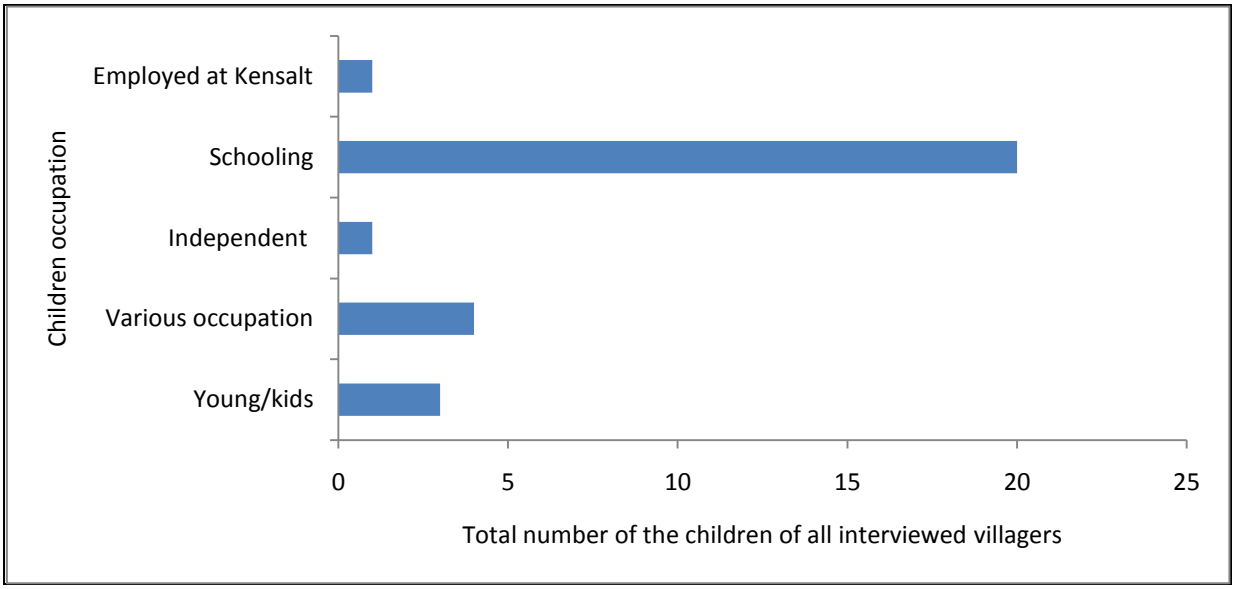


Figure 4. 16 Children's occupation in Kadzuhoni community

Religious belief

The people who believe neither Christian nor Islamic religions ranked the top in the community (53 %). Christian believers became the second (30 %) and the least were the Islam believers (Figure 4.17).

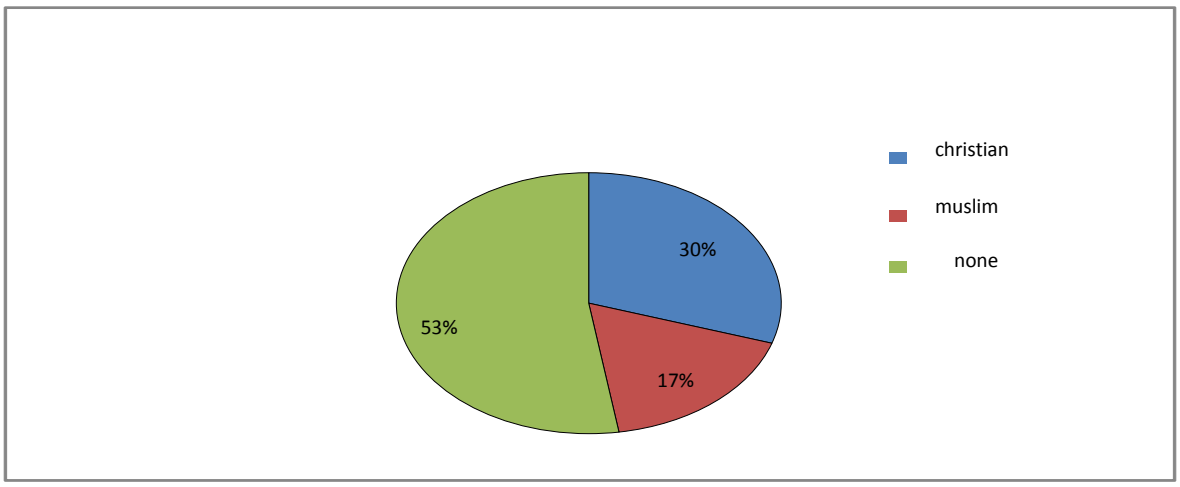


Figure 4. 17 Religious convictions in Kadzuhoni community

Education level

Many people had not completed primary school level (19 people); people who had not gone to school ranked the second in the community. About 12 people amongst the interviewees had not gone to school. Also 3 of them had not been able to complete primary education while 3 did not complete secondary education. Amongst all people interviewed, only 1 had a diploma training certificate and none had attended University. Lastly 1 person completed primary education and 1 had primary education and a course certificate (Figure 4.18).

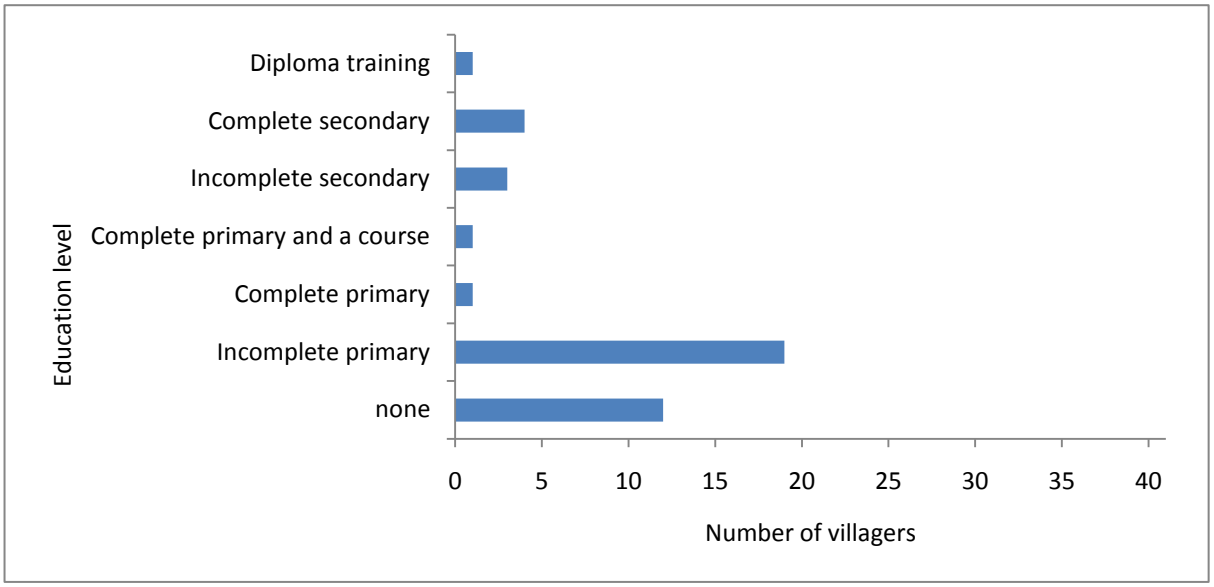


Figure 4. 18 Education levels of the Kadzuhoni community

Interviewee occupation

Highest number of the villagers (11) interviewed was employed by Kensalt Company. This was then followed by the farmers and business men (8). Fishers (8) who went to the ocean day and night for fishing activities ranked the third group of people in the community. Also, the community consisted of the old people; women were busy with taking care of their families and students (Figure 4.19).

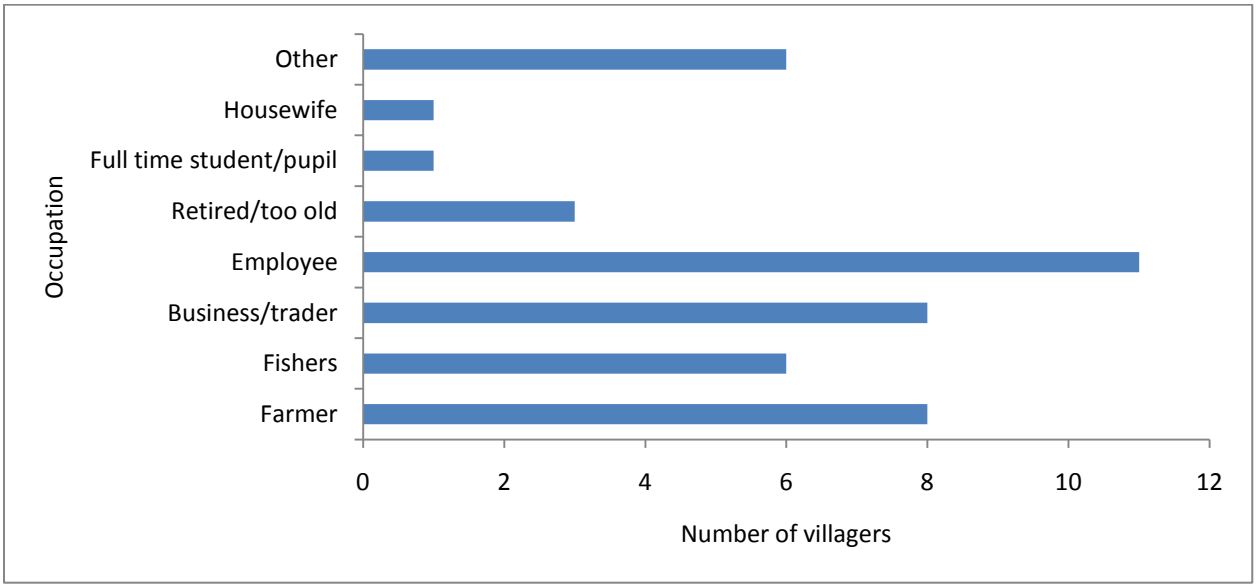


Figure 4. 19 Interviewee occupation

4.3 Laboratory experiments

4.3.1 Effect of temperature on survival, growth and reproduction

4.3.1.1 Survival

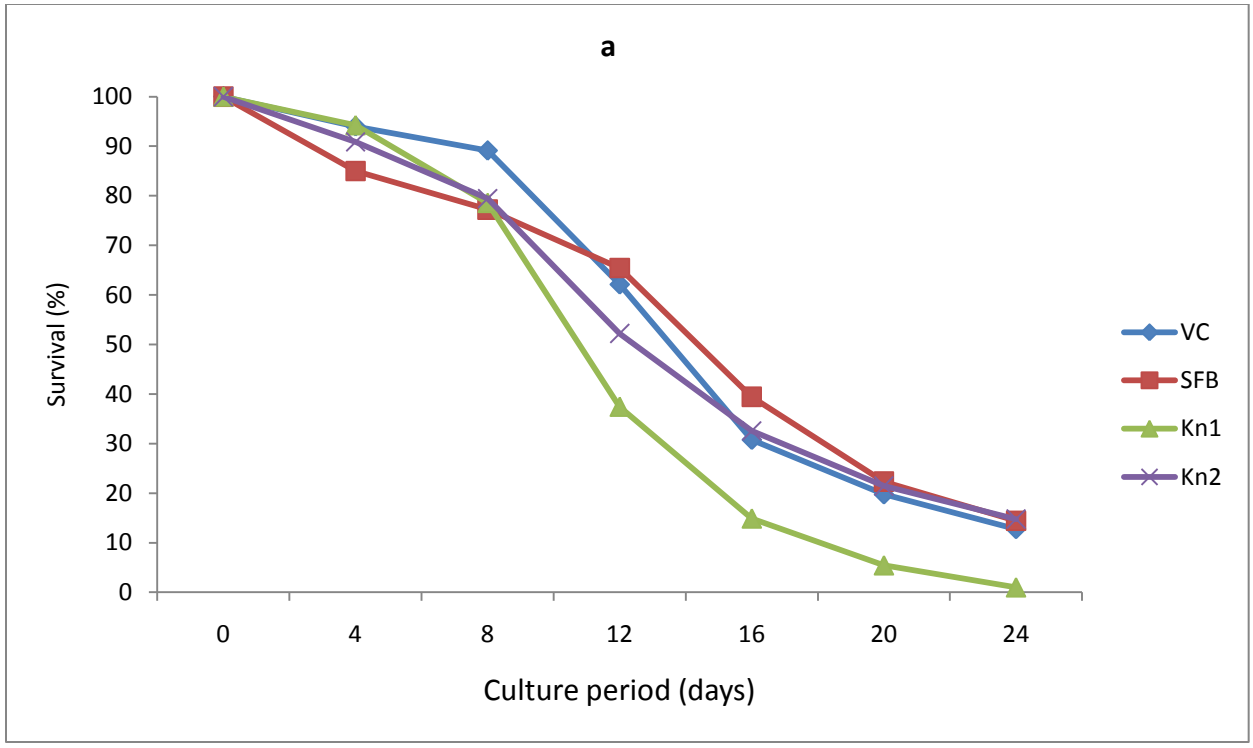
Table 4.8 depicts the mean survival of the experimental strains for the entire culture period at 28, 32 and 36°C. Generally, survival decreased as the temperature increased (Figure 4.20). A high percentage of animals surviving was found at 28°C before day 12 followed by those animals exposed at 32°C. The lowest percentage of survivors was found at 36°C. At 28°C, the survival for all strains was above 77% up to the end of day 8; VC strain was leading by 89%. As from day 8 up to the end of the culture period drastic mortalities were observed for all strains at 28°C but Kn1 showed the lowest survival (Figure 4.20a). At 32°C, drastic mortalities were recorded from day 4 up to the end of the culture period and at this temperature SFB strain displayed the lowest survival from day 0 onwards. At 36°C, Kn2 showed less low survival (21%) than others while SFB indicated the lowest survival (Figure 4.20c).

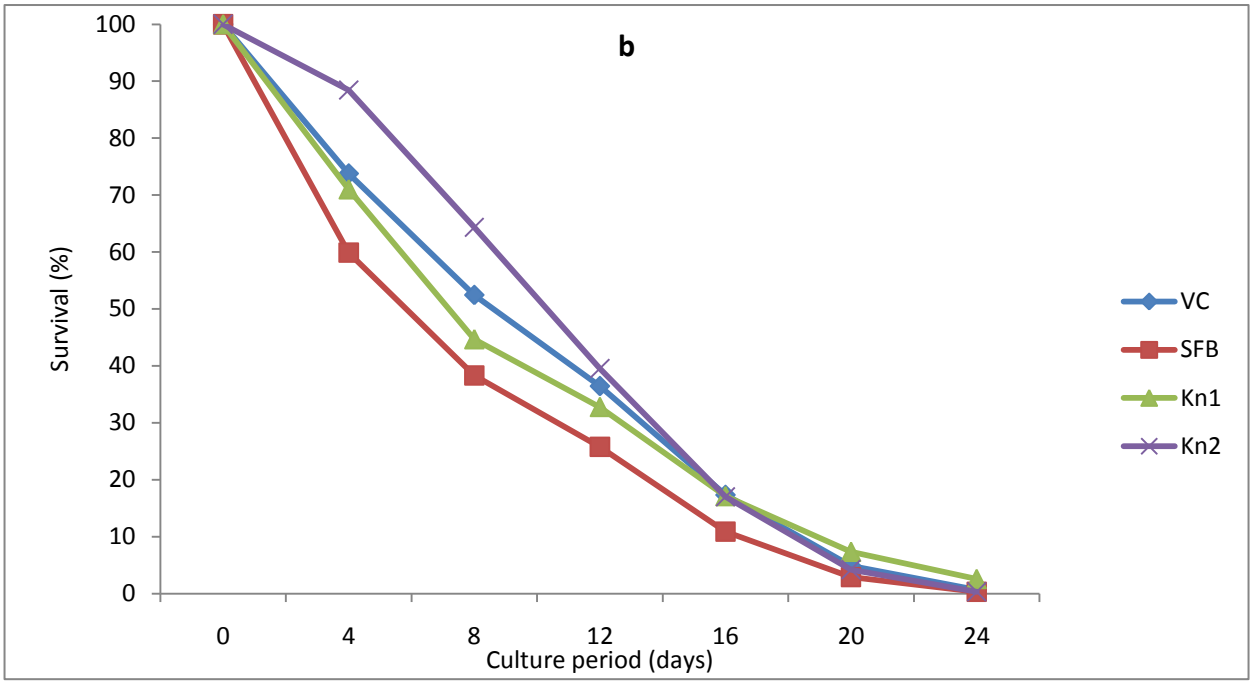
Statistical analysis for the effect of strain indicated that at 28°C there was no significant difference in survival after day 4 and 8 of the culture periods although SFB had a little bit lower survival (85% and 77% respectively) compared to other strains. At day 16 of the culture period Kn1 had significantly lower survival (15%) compared to SFB (39%) and Kn2 (33%). The results for survival at day 4 and 8 at 32°C showed that the survival of VC and Kn1 were similar, while SFB indicated significantly lower ($p<0.05$) survival than Kn2, and while there no significant differences for the rest of the culture period at this temperature.

At 36°C, there was no significant ($p>0.05$) difference in survival detected between all strains both in days 4 and 8. At this temperature all animals of SFB strain had died by day 4 while the rest had died by day 8.

For the effect of temperature, all strains cultured showed significantly ($p<0.05$) lower survival at 36°C than 28 and 32°C in day 4. In day 8, all animals cultured at 28°C were shown to have significantly ($p<0.05$) higher survival than 32 and 36°C, except for Kn2 which did not show any significant difference between 28 and 32°C. In day 12 the only significant difference found was in SFB having lower survival at 32°C than at 28°C. In days 16, 20 and 24, VC and SFB were shown to have significantly ($p<0.05$) higher survival at 28°C than at 32°C. Kn2 strain indicated a significantly ($p<0.05$) higher survival at 28°C than at 32°C in both days 20 and 24.

Two-way ANOVA analysis of the survival data indicated a significant ($p<0.05$) interaction between the strain and the temperature after day 12 up to the end of the culture period (Table 4.9).


a
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Culture period (days)
VC
SFB
Kn1
Kn2


b
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Culture period (days)
VC
SFB
Kn1
Kn2

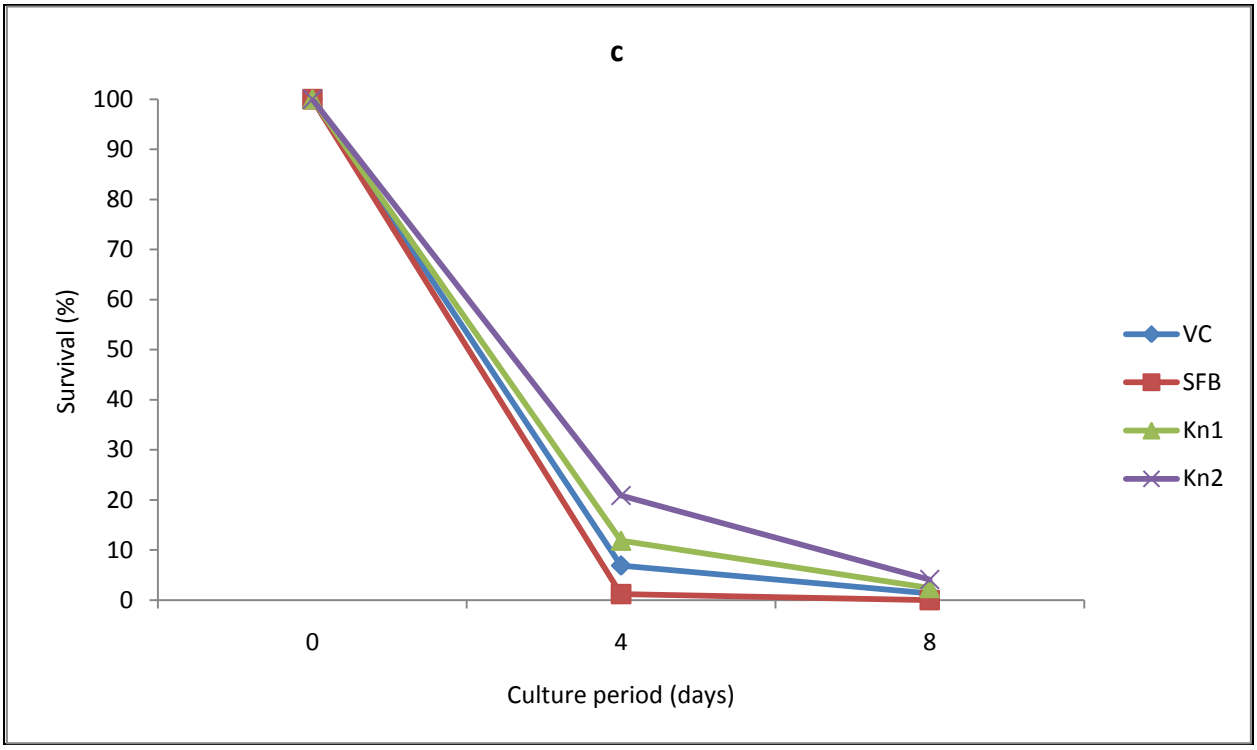


Figure 4. 20a, b and c Mean survival of the experimental *Artemia* strains at 28°C (a), 32°C (b) and 36°C (c) for the entire culture period

Table 4. 8 Survival (%) for the entire culture period at 28, 32 and 36°C. Data present mean and standard deviation of the 3 replicates. Values on the same row sharing the same superscript are not significantly different (one-way ANOVA, p>0.05)

4.8a One-way ANOVA: effect of strain

Strain	VC	SFB	Kn1	Kn2
28°C				
Survival day 4	94.0±1.3 ^{a}	85.0±6.7^{a}	94.2±2.5 ^{a}	90.9±10.3^{a}
Survival day 8	89.1±1.6 ^{a}	77.2±6.4^{a}	78.6±2.5 ^{a}	79.4±9.1^{a}
Survival day 12	62.1±14.3^{b}	65.4±6.5^{b}	37.4±7.4 ^{a}	52.2±6.5^{ab}
Survival day 16	30.8±6.7^{ab}	39.4±5.32^{b}	14.9±3.3 ^{a}	32.6±9.^{b}
Survival day 20	19.8±7.0^{a}	22.3±9.3^{a}	5.4±1.8 ^{a}	21.4±8.3^{a}
Survival day 24	12.8±7.1 ^{a}	14.4±9.1 ^{a}	1.0±1.5 ^{a}	14.8±6.3^{a}
32°C				
Survival day 4	73.8±14.8^{ab}	59.9±8.4^{a}	71.0±7.6^{ab}	88.4±7.8^{b}
Survival day 8	52.4±15.1^{ab}	38.3±6.6^{a}	44.7±1.0^{ab}	64.3±8.5^{b}
Survival day 12	36.4±10.7^{a}	25.8±3.60^{a}	32.8±3.^{a}	39.6±9.8^{a}
Survival day 16	17.3±5.5^{a}	10.9±3.8^{a}	17.1±2.8^{a}	17.0±6.2^{a}
Survival day 20	4.9±2. ^{a}	2.9±2.5^{a}	7.3±2.7^{a}	4.2±2.9^{a}
Survival day 24	0.7±0.9^{a}	0.3±0.3^{a}	2.6±2.2^{a}	0.3±0.3^{a}
36°C				
Survival day 4	6.9±8.5^{a}	1.2±1.6^{a}	11.9±10.7^{a}	20.9±2.0^{a}
Survival day 8	1.3±2.3^{a}	0.0±0.0^{a}	2.4±4.2^{a}	4.1±3.8^{a}

4.8b One way ANOVA: effect of temperature

	Survival at day 4			Survival at day 8		
Strain	28°C	32°C	36°C	28°C	32°C	36°C
VC	94.0±1.3[b]	73.8±14.8[b]	6.9±8.5[a]	89.1±1.6[c]	52.4±15.1[b]	1.3±2.3[a]
SFB	85.0±6.7[b]	59.9±8.4[b]	1.2±1.6[a]	77.2±6.4[c]	38.3±6.6[b]	0.0±0.0[a]
Kn1	94.2±2.5[b]	71.0±7.6[b]	11.9±10.7[a]	78.6±2.5[c]	44.7±1.0[b]	2.4±4.2[a]
Kn2	90.9±10.3[b]	88.4±7.8[b]	20.9±19.0[a]	79.4±9.1[b]	64.3±8.5[b]	4.1±3.8[a]

Table continued

Survival at day 12		Survival at day 16		Survival at day 20		Survival at day 24	
28°C	32°C	28°C	32°C	28°C	32°C	28°C	32°C
62.1±14.0[a]	36.4±10.7[a]	30.8±6.7[b]	17.3±5.5[a]	19.8±7.0[b]	4.9±2.8[a]	12.8±7.1[b]	0.7±0.9[a]
65.4±6.5[b]	25.8±3.6[a]	39.4±5.3[b]	10.9±3.8[a]	22.3±9.3[b]	2.9±2.5[a]	14.4±9.1[b]	0.3±0.3[a]
37.4±7.4[a]	32.8±3.10[a]	14.9±3.3[a]	17.1±2.8[a]	5.4±1.8[a]	7.3±2.7[a]	1.0±1.5[a]	2.6±2.2[a]
52.2±6.5[a]	39.6±9.8[a]	32.6±9.1[a]	17.0±6.2[a]	21.4±8.3[b]	4.2±2.9[a]	14.8±6.3[b]	0.3±0.3[a]

Table 4. 9 Effect of strain and temperature on *Artemia* survival (p-values, two-way ANOVA); an asterisk denotes a significance at p=0.05

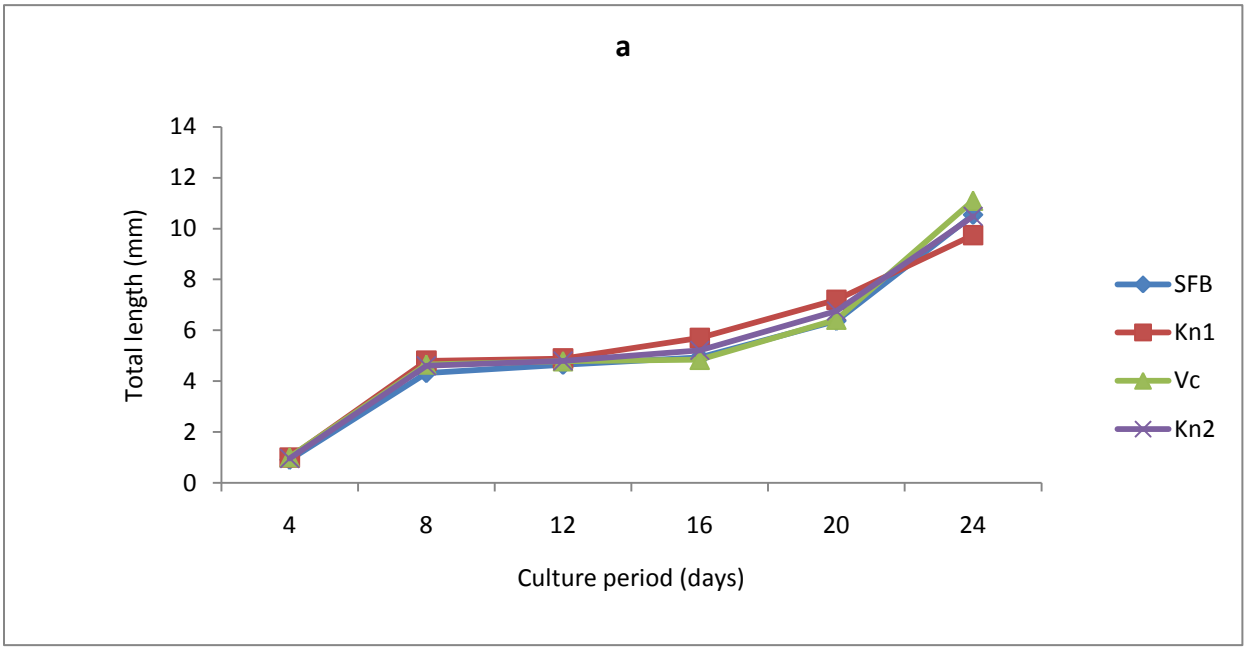
Survival parameter	**day 4**	**day 8**	**day 12**	**day 16**	**day 20**	**day 24**
Effect of temperature	0.0016*	0.0003*	0.0023*	0.0032*	0.0121*	0.0178*
Effect of strain	0.0441*	0.0261*	0.0792	0.0536	0.1135	0.0997
Interaction	0.1788	0.0903	0.0243*	0.0053*	0.0098*	0.0174*

4.3.1.2 Growth

The mean length of the different strains of *Artemia* for day 0 to 24 of the culture period cultured at 28 and 32°C is plotted in Figure 4.21a and 4.21b. From day 4 to 8 of the culture period all strains grew fast at both temperatures (28 and 32°C), slower from day 8 to 16, followed by a faster growth again from day 16 to 24 at 28°C. At 32°C, slow growth was observed over a long time (from day 8 to day 20), but then the animals showed an increased growth from day 20 to day 24. The animals cultured at 32°C (Figure 4.21b) showed somewhat higher length at day 24 (11.8 mm, VC strain) than at 28°C (11.1 mm) (Figure 4.21a).

One-way ANOVA of the analysis of the effect of strain showed that, at 28°C no significant difference in length among the strains was observed after day 4 to 12 of the culture period. In day 16, Kn1 had a significantly higher length compared to other strains while in day 20 Kn1 strain had a significantly ($p<0.05$) higher (7.2 mm) length than VC and SFB. In the last day of the culture period VC showed a significantly ($p<0.05$) higher length (11.1 mm) compared to Kn1. At 32°C, no significant differences were shown in days 4, 16 and 20. After days 8 and 12, SFB strain indicated a significantly ($p<0.05$) slower growth compared to the other strains. In day 24 VC showed a significantly ($p<0.05$) higher length compared to Kn1 and SFB strains (Table 4.10). Analysis of the effect of temperature shows that at 36°C all surviving strains have a significantly ($p<0.05$) smaller length than at 32 and 28°C in day 8. At day 20, both Kenyan strains showed a significantly ($p<0.05$) higher length at 28°C than at 32°C.

A two factor ANOVA analysis for the length data showed significant interaction between strain and temperature ($p<0.05$) at day 8, 16 and 24 days of the culture period (Table 4.11).



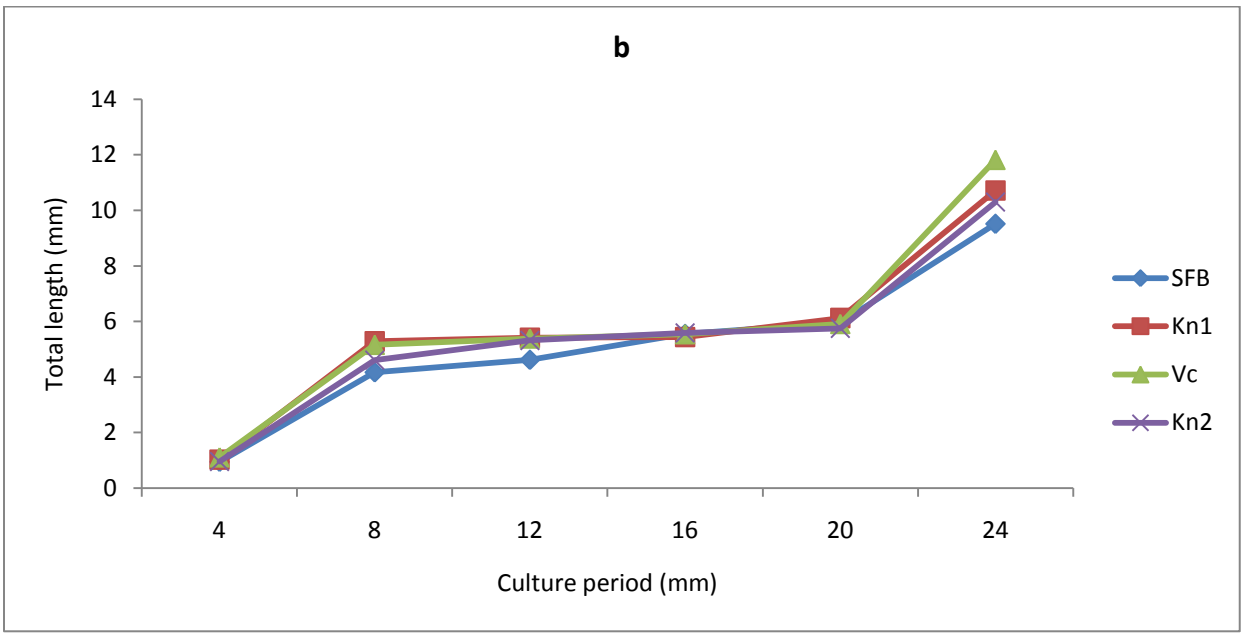


Figure 4. 21a and b Mean length of the experimental *Artemia* strains at 28°C (a) and 32°C (b) for the entire culture period

Table 4. 10 Length (mm) over the entire culture period at 28, 32 and 36°C. Data present mean and standard deviation of 30 animals per temperature per population. Values on the same row sharing the same superscript are not significantly different (One-way ANOVA, p>0.05).

4.10a One way ANOVA: effect of strain

	SFB	Kn1	VC	Kn2
28°C				
Length day 4	0.9±0.1^{a}	1.0±0.2^{a}	1.0±0.2^{a}	1.0±0.1^{a}
Length day 8	4.3±0.9^{a}	4.8±1.0^{a}	4.7±0.8^{a}	4.6±0.8^{a}
Length day 12	4.7±0.7^{a}	4.9±0.6^{a}	4.8±0.6^{a}	4.8±0.8^{a}
Length day 16	4.9±0.5^{a}	5.7±0.9^{b}	4.9±0.6^{a}	5.2±1.0^{a}
Length day 20	6.4±0.9^{a}	7.2±1.1^{b}	6.4±1^{a}	6.8±0.8^{ab}
Length day 24	10.5±1.2^{ab}	9.7±2.2^{a}	11.1±1.5^{b}	10.5±1.3^{ab}
32°C				
Length day 4	1.0±0.1^{a}	1.0±0.2^{a}	1.1±0.1^{a}	1.0±0.1^{a}
Length day 8	4.2±1.0^{a}	5.3±0.9^{b}	5.2±1.0^{b}	4.6±0.7^{b}
Length day 12	4.6±0.7^{a}	5.4±0.9^{b}	5.4±0.9^{b}	5.3±1.1^{b}
Length day 16	5.6±1.3^{a}	5.4±1.6^{a}	5.5±1.0^{a}	5.6±0.9^{a}
Length day 20	6.0±0.6^{a}	6.1±0.7^{a}	5.9±0.6^{a}	5.8±0.6^{a}
Length day 24	9.5±2.1^{a}	10.7±1.5^{ab}	11.8±2.3^{b}	10.3±1.8^{ab}
36°C				
Length day 4	0.7±0.1^{a}	0.8±0.2^{a}	0.7±0.1^{a}	0.8±0.1^{a}
Length day 8	-	2.3± 0.5^{a}	3.1±0.0^{a}	2.3±0.2^{a}

4.10b One way ANOVA: Effect of temperature

Strain	day 4			day 8			day 12	
	28°C	32°C	36°C	28°C	32°C	36°C	28°C	32°C
SFB	0.9±0.1^{a}	1.0±0.1^{a}	0.7±0.1^{a}	4.3±0.9^{a}	4.2±1.0^{a}	-	4.7±0.7^{a}	4.7±0.7^{a}
Kn1	1.0±0.2^{a}	1.0±0.2^{a}	0.8±0.2^{a}	4.8±1.0^{b}	5.3±0.9^{b}	2.3±0.5^{a}	4.9±0.6^{a}	5.4±0.9^{a}
VC	1.0±0.2^{a}	1.1±0.1^{a}	0.7±0.1^{a}	4.7±0.8^{b}	5.2±1.0^{b}	3.1±0^{a}	4.8±0.6^{a}	5.4±0.9^{a}
Kn2	1.0±0.1^{a}	1.0±0.1^{a}	0.8±0.1^{a}	4.6±0.8^{b}	4.6±0.7^{b}	2.3±0.2^{a}	4.8±0.8^{a}	5.3±1.1^{a}

Table continued

	day 16		day 20		day 24	
Strain	28°C	32°C	28°C	32°C	28°C	32°C
SFB	4.9±0.5^{a}	5.6±1.3^{b}	6.4±0.9^{a}	6.0±0.6^{a}	10.5±1.2^{a}	9.5±2.1^{a}
Kn1	5.7±0.9^{a}	5.4±1.6^{a}	7.2±1.1^{b}	6.1±0.7^{a}	9.7±2.2^{a}	10.7±1.5^{a}
VC	4.8±0.1^{a}	5.5±1.0^{a}	6.4±1.0^{a}	5.9±0.6^{a}	11.1±1.5^{a}	11.8±2.3^{a}
Kn2	5.2±1.0^{a}	5.6±0.9^{a}	6.8±0.8^{b}	5.8±0.6^{a}	10.5±1.3^{a}	10.3±1.8^{a}

Table 4. 11 Effect of strain and temperature on *Artemia* length (p-values, two-way ANOVA); an asterisk designates a significance at p= 0.05

Length parameter	day 4	day 8	day 12	day 16	day 20	Day 24
Effect of temperature	0.1686	0.9871	0.0114*	<0.0001*	<0.0001*	<0.0001*
Effect of strain	0.0374*	0.0527	0.0049*	0.0810	0.0584	0.1826
Interaction	0.2880	0.0056*	0.2283	0.0011*	0.0519	0.0244*

4.3.1.3 Reproductive characteristics

Female pre-reproductive period

The longest pre-reproductive period was recorded in SFB strain (25.1 days) at 28°C. At 32°C, Kn1 showed the longest pre-reproductive period (16.3 days) followed by both Kn2 and SFB (15.9 days) and the lowest value was in VC. The overall results showed that at low temperature Kn1 showed shorter pre-reproductive period while at high temperature (32°C) it had longer pre-reproductive period compared to the other strains. On the other hand, Kn2, VC and SFB revealed shorter reproductive period with increase in temperature compared to Kn1 (Table 4.13a).

One-way ANOVA analysis of the effect of strain showed that at 28°C the pre-reproductive period of SFB was significantly ($p<0.05$) longer than for Kn1 and Kn2; Kn1 had significantly shorter pre-reproductive period than VC and SFB at this temperature. At 32°C Kn1 showed a significantly ($p<0.05$) longer pre-reproductive period compared to VC strain (Table 4.12). Unlike other strains, Kn1 had significantly ($p<0.05$) shorter period at 28°C (14.1 days) than at 32°C ($p<0.05$) while SFB and VC strains had a significantly longer pre-reproductive period at 28°C than at 32°C.

A two-way ANOVA test for the pre-reproductive period divulged that there was significant interaction between temperature and strain ($p<0.0001$) (Table 4.13).

Female reproductive period

For this parameter, VC had significantly ($p<0.05$) longer reproductive period (12.6 days) than Kn2 at 28°C. The order of the increase in the reproductive period at 28°C was VC>Kn1>SFB>Kn2 while at 32°C was Kn2>VC>Kn1>SFB. Analysis of the effect of temperature showed that Kn1, VC and SFB had a significantly ($p=0.05$) longer reproductive period at 28°C than at 32°C.

A two factor ANOVA revealed a significant ($p=0.0064$) interaction between temperature and strain for the reproductive period (Table 4.13).

Total broods per female

At 28°C VC showed the highest total number of broods (2.9) while Kn1 dominated at 32°C (1.1). The order of broods size at 28°C was VC>SFB>Kn2>Kn1 and at 32°C Kn1>VC>Kn2>SFB (Table 4.12).

The total number of broods was not significant difference ($p>0.05$) at both temperatures. As for the effect of temperature, both VC and SFB had more broods at 28°C than at 32°C but not significantly ($p>0.05$) difference.

Two factor ANOVA did not reveal a significant (p=0.3311) interaction between strain and temperature (Table 4.13).

Oviparous broods per female

There was no any significant (p>0.05) difference in oviparous broods per female for all strains. Generally low number of oviparous broods (0.1 – 0.4) was released at both temperatures; SFB reproduced solely ovoviviparously at 32°C (Table 4.12). Likewise, analysis of temperature effect did not show any significant difference in broods at both temperatures.

A two-way ANOVA did not detect any interactive effect (p=0.1375) for oviparous broods per female (Table 4.13).

Ovoviviparous broods per female

At 28°C strains showed the values in the following order: VC>SFB>Kn1=Kn2. Generally, more broods were observed at 28°C than at 32°C (Table 4.12).

VC had higher number of ovoviviparous broods (2.5) compared to the other strains at 28°C although no significant (p>0.05) difference was detected. Also, analysis for the effect of temperature did not show any significant difference.

There was no significant interaction for this parameter detected between strain and temperature using a two-way ANOVA (p=0.3649) (Table 4.13).

Total offspring per female

Reproductive performance followed the order VC>SFB>Kn1>Kn2 at 28°C and Kn2>VC>Kn1>SFB at 32°C. At 28°C the highest number of total offspring per female was recorded for the VC strain (210 individuals/female; the value was significantly higher than for the Kenyan strains) while the lowest number was 49.7 individuals/female for Kn2. At 32°C the Kn2 strain had high number of offspring per female (67.8 individuals/female). Generally all strains (with the exception of Kn2) cultured at 28°C produced higher total offspring compared to 32°C. As for the effect of strain, at 28°C Kn2 had a significantly (p<0.05) lower total number of offspring than VC while at 32°C the SFB strain produced significantly (p<0.05) lower number of offspring than Kn2 and VC (Table 4.12). According to the comparison of the effect of temperature, Kn1 and VC had significantly (p<0.05) higher total number of offspring than at 32°C, while the opposite was the case for Kn2 (Table 4.12).

Two way ANOVA showed significant (p=0.0039) interaction between the strain and the temperature for this parameter (Table 4.13).

Offspring per female per day

At 28°C, the order of the number of offspring per female per day was Kn1=VC>Kn2>SFB. At 32°C the order was: Kn1>VC>Kn2>SFB.

SFB was shown to have significantly (p<0.05) lower offspring per female per day than VC and Kn1 at 28°C and than VC, Kn1 and Kn2 at 32°C (Table 4.12). Analysis of the effect of temperature showed that at 28°C Kn1 and VC had significantly (p<0.05) more offspring per day than at 32°C.

There was no a significant (p=0.1839) interaction between strain and temperature detected by a two-way ANOVA (Table 4.13).

Percent offspring encysted

The order of the number of the encysted offspring was: Kn2=SFB>VC>Kn1 at 28°C. At 32°C the order was: Kn1>Kn2>VC>SFB. Generally, all strains exposed at 32°C showed high percentage of encysted offspring compared to 28°C (except for SFB). As for the effect of strain, SFB showed significantly ($p<0.05$) lower percentage offspring encysted than the Kenyan strains at 32°C. Analysis of the effect of temperature showed that all Kenyan and VC strains had significantly ($p<0.05$) higher percentage offspring encysted at 32°C than at 28°C while the opposite was the case for SFB (Table 4.12).

By two factor ANOVA analysis for percentage offspring encysted, no significant ($p=0.0890$) interaction between the strain and the temperature was revealed (Table 4.13).

Brood interval

All strains at 28°C indicated similar values for this characteristic. The shortest brood interval found was 1.1 days (SFB at 32°C) and the longest was 2.4 days (Kn2 at 32°C).

Analysis of the effect of strain showed that, at 32°C a significantly ($p<0.05$) longer brood interval was recorded for Kn2 compared to VC and SFB. Analysis of temperature effect showed that both SFB and VC had significantly longer brood interval at 28°C than 32°C (Table 4.12).

The interaction between the strain and temperature was significant (two-way ANOVA, $p=0.0009$) for this parameter (Table 4.13)

Table 4. 12 Reproductive and lifespan characteristics for experimental *Artemia* strains at 28, 32 and 36°C. Mean of 30 females per temperature per population. For each temperature, values on the same row sharing the same superscript are not significantly different (ANOVA, p>0.05).

4.13a One way ANOVA: effect of strain

Reproductive Characteristics	A.28° C				B.32 °C			
	KN1	KN2	VC	SFB	KN1	KN2	VC	SFB
Female pre-reproductive period (days)	14.1[a] (1.7)	16.9[ab] (1.4)	19.1[bc] (3.8)	25.1[c] (4.0)	16.3[b] (3.2)	15.9[ab] (2.5)	15.2[a] (2.3)	15.9[ab] (2.8)
Female reproductive period (days)	9.9[ab] (6.7)	4.8[a] (3.8)	12.6[b] (7.9)	9.6[ab] (4.0)	4.6[ab] (3.9)	5.9[b] (3.9)	4.8[ab] (3.6)	2.3[a] (1.5)
Total broods per female	0.9[a] (1.5)	1.0[a] (1.5)	2.9[a] (3.3)	2.2[a] (2.3)	1.1[a] (1.2)	0.6[a] (1.1)	0.9[a] (1.4)	0.3[a] (0.6)
Oviparous broods per female	0.1[a] (0.2)	0.1[a] (0.2)	0.4[a] (0.7)	0.3[a] (0.6)	0.1[a] (0.3)	0.1[a] (0.4)	0.2[a] (0.5)	0.0[a] (0.0)
Ovoviviparous broods per female	0.9[a] (1.4)	0.9[a] (1.1)	2.5[a] (2.8)	1.9[a] (2.1)	1.0[a] (1.1)	0.4[a] (0.8)	0.7[a] (1.0)	0.3[a] (0.6)
Total offspring per female	98.0[a] (93.0)	49.7[a] (56.4)	210.0[b] (186.0)	143.0[ab] (98.1)	39.0[ab] (35.0)	67.8[b] (62.6)	52.0[b] (54.0)	14.0[a] (21.0)
Offspring per female per day	12.0[b] (7.6)	7.6[ab] (8.5)	12.0[b] (6.3)	3.8[a] (1.8)	6.8[b] (3.5)	6.3[b] (5.6)	6.6[b] (6.4)	1.9[a] (2.3)
Percent offspring encysted	2.0[a] (7.0)	11.0[b] (31.0)	3.0[a] (5.0)	11.0[b] (25.0)	75.0[b] (49.0)	52.1[b] (41.7)	18.3[ab] (31.8)	0.0[a] (0.0)
Brood interval (days)	1.6[a] (1.0)	1.3[a] (0.8)	2.2[a] (1.1)	2.3[a] (0.9)	1.8[ab] (1.4)	2.4[b] (1.2)	1.4[a] (0.6)	1.1[a] (0.4)

4.13b One way ANOVA: effect of temperature

Strains	Female pre-reproductive period* (days)		Female reproductive period (days)		Total broods per female		Oviparous broods per female	
	28°C	32°C	28°C	32°C	28°C	32°C	28°C	32°C
KN1	14.1[a] (1.7)	16.3[b] (3.2)	9.9[b] (6.7)	4.6[a] (3.9)	0.9[a] (1.5)	1.1[a] (1.2)	0.1[a] (0.2)	0.1[a] (0.3)
KN2	16.9[a] (1.4)	15.9[a] (2.5)	4.8[a] (3.8)	5.9[b] (3.9)	1.0[a] (1.5)	0.6[a] (1.1)	0.1[a] (0.2)	0.1[a] (0.4)
VC	19.1[b] (3.8)	15.2[a] (2.3)	12.6[b] (7.9)	4.8[a] (3.6)	2.9[a] (3.3)	0.9[a] (1.4)	0.4[a] (0.7)	0.2[a] (0.5)
SFB	25.1[b] (4.0)	15.9[a] (2.8)	9.6[b] (4.0)	2.3[a] (1.5)	2.2[a] (2.3)	0.3[a] (0.6)	0.3[a] (0.6)	0.0[a] (0.0)

Table continued

	Ovoviviparous broods per female		Total offspring per female		Offspring per female per day		Percent offspring encysted		Brood interval (days)	
Strains	28°C	32°C	28°C	32°C	28°C	32°C	28°C	32°C	28°C	32°C
KN1	0.9[a]	1.0[a]	98.0[b]	39[a]	12.0[b]	6.8[a]	2.0[a]	75.0[b]	1.6[a]	1.8[a]
	(1.4)	(1.2)	(93.0)	(35.0)	(7.6)	(3.5)	(7.0)	(49.0)	(1.0)	(1.4)
KN2	0.9[a]	0.4[a]	49.7[a]	67.8[b]	7.6[a]	6.3[a]	11.0[a]	52.1[b]	1.3[a]	2.4[a]
	(1.2)	(0.9)	(56.4)	(62.6)	(8.5)	(5.6)	(31.0)	(41.7)	(0.8)	(1.2)
VC	2.5[a]	0.7[a]	210.0[b]	52.0[a]	12.0[b]	6.6[a]	3.0[a]	18.3[b]	2.2[b]	1.4[a]
	(2.8)	(1.0)	(186.0)	(54.0)	(6.3)	(6.4)	(5.0	(31.8)	(1.1)	(0.6)
SFB	2.1[a]	0.3[a]	143.0[b]	14.0[a]	3.8[a]	1.9[a]	11.0[b]	0.0[a]	2.3[b]	1.1[a]
	(1.8)	(0.7)	(98.1)	(21.0)	(1.8)	(2.3)	(25.0)	(0.0)	(0.9)	(0.4)

Table 4. 13 Effect of strain and temperature on *Artemia* length (p-values, two-way ANOVA, P=0.05); an asterisk denotes significance

Reproductive parameter	**Effect of temperature**	**Effect of strain**	**Interaction**
Pre-reproductive period	0.0215*	0.0520	<0.0001*
Reproductive period	0.0001*	0.0054*	0.0064*
Total offspring per female	<0.0001*	0.0044*	0.0039*
Oviparous brood	0.1894	0.4436	0.1375
Percentage offspring encysted	0.3350	0.8296	0.0890
Brood interval	0.0083*	0.7059	0.0009*
Ovoviviparous brood	0.0500	0.3250	0.3649
Offspring per female per day	0.0001*	0.0834	0.1839
Total broods per female	0.0632	0.3348	0.3311

4.3.2 Thermotolerance test

The highest survival after the heat shock was shown by Kn2 (79%), followed by VC (65%), Kn1 (45%) and the lowest one was SFB (13%) (Figure 4.22).

One way ANOVA revealed a significant difference in survival between the strains: SFB was shown to have significantly lower survival ($p<0.001$) compared to Kn2 and Kn1. Kn2 showed significantly ($P<0.05$) higher survival than Kn1 and SFB.

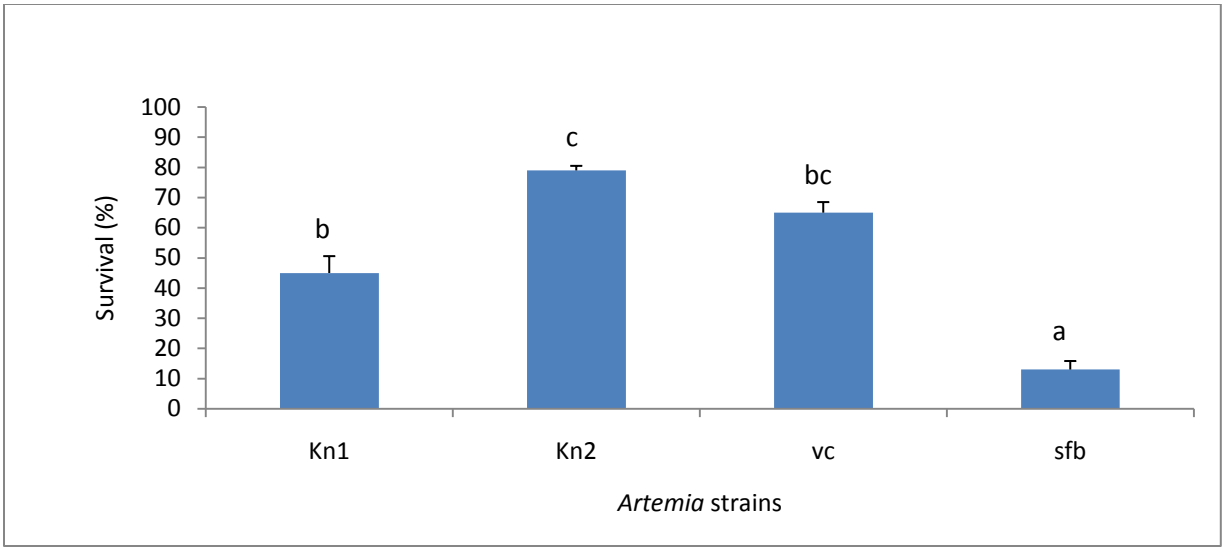


Figure 4. 22 Survival (%) for heat shock experiment of 4 different *Artemia* strains, error bars are standard deviation; points are a mean of 3 replicates. Bars sharing the same superscript are not significantly different ($p>0.05$).

5 DISCUSSION

5.1 Field work

5.1.1 Physico-chemical parameters

In our study at Malindi, mean water temperatures fluctuated from approximately 30°C in the morning to as high as 39°C in the afternoon and then dropped to 36°C in the evening for the culture ponds (Table 4.1.). In consequence to this variation, *Artemia* were stressed, hiding along the trenches, and frequent reddening in colour of the animals was observed in the culture ponds. Anh et al. (2009) accounted for the variation of temperatures of 22 – 29°C in the morning and high temperature of 37°C in the afternoon. Furthermore, Lulijwa (2010) while working in the Mekong delta reported mean temperature fluctuations of 27 – 37°C between the morning and afternoon respectively.

Thoeye et al. (1987) examined the effect of steady and recurring temperature regimes ranging from 20 – 35°C on survival of *Artemia franciscana* and reported better temperature tolerance under unstable temperatures compared to a regime of constant temperatures. They also reported that the level of tolerance increase is reduced with large differences between the two temperature extremes. According to Vos (1979), the optimal temperature for the growth of *Artemia* in Southeast Asia is 28°C while higher temperatures of 36 – 37°C kill *Artemia.*

In our work, mean weekly salinity varied from 99 g/L to 119 g/L, which is a higher range than those reported by Anh et al. (2009) (75 – 114 g/L) and Lulijwa (2010) (76 – 92 g/l) in similar culture experiments. Significantly ($p<0.05$) higher salinity was recorded in the control culture ponds than in the fertilized culture ponds for the entire culture period (Table 4.1). The drop in salinity in the fertilized culture ponds was caused by the lack of expertise or inadequate infrastructure. Lulijwa (2010) reported late maturation in his experiment due to low salinity. Contrarily, in our study early maturation was observed in the fertilized treatment with a mean weekly salinity of 99 g/L which is lower than the salinity in the control (119 g/L). Likewise, Basil and Pandian (1991) documented that the variation of salinity in culture systems tends to affect the *Artemia* population in terms of survival although nauplii are known to survive sudden changes in salinity.

Our study indicated a turbidity fluctuation in the control ponds from 18 cm in week 1, possibly due to algal blooms resulting from poor filtration by *Artemia* nauplii, to an average of 22 cm in week 3 when filter feeding was efficient in adults. In the fertilized treatment, mean turbidity in week 1 was 21 cm and went down to as low as 18 cm in week 4, possibly due to low levels of water in the ponds caused by high seepage and evaporation rate (4 cm per day). The condition of high turbidity in the control led to the increased risk of reduced oxygen in the water column causing stress to *Artemia*. Anh et al. (2009) reported that a suitable turbidity range for the optimal survival of *Artemia* should be around 25 to 30 cm.

5.1.2 Nutrient parameters

In general, low nutrient (phosphate, nitrate and ammonia) concentrations were observed in the creeks and the culture ponds during the first and the second months of the culture periods followed by higher levels at the end of the culture period (third month). The same observation was reported by Anh (2009) while working at Vinh Chau, Vietnam.

The low phosphate concentration in the ponds during the 1st and 2nd months of the culture period could be due to high abundance of phytoplankton in the culture system which leads to phosphate assimilation by the algae. Also, Masuda and Boyd (1994) reported that a high amount of phosphorus is absorbed by the soil reducing its availability for the phytoplankton. During the 3rd month, the concentration of phosphate was significantly high probably due to high levels from the source.

Also the low nitrate concentration in all culture ponds and the creeks during the first two months of the culture period could be due to the assimilation by algal blooms, while its increase during the third month could probably be due to high levels from the source. Boyd (2000) explained that aquatic plants absorb nitrogen added to the water and deposit it on the soil as a constituent of the organic matter. In addition, very high levels of inorganic nitrogen are denitrified in the mud and water while another fraction gets lost through ammonium volatilization in the atmosphere hence becoming unavailable to the phytoplankton in the ponds (Boyd, 1990).

Very low levels of ammonia in the creeks and all culture ponds during the 1st and 2nd month suggest high removal by bacteria and high absorption by algae. Generally higher levels of ammonia were found in all creeks and culture ponds during the third month than during the first and second months. Probably high mortality of *Artemia* and their decomposition in the control culture ponds could have contributed to these higher levels of ammonia observed.

5.1.3 Phytoplankton and non-*Artemia* zooplankton

In our study, various genera/species of phytoplankton and zooplankton were found. Both beneficial and non-beneficial algae were found and dominated the species composition at different times during the culture period. Some species were showing up for the entire culture period as they were favoured by high salinity (over 100 g/L). Higher phytoplankton abundance was recorded in the non-fertilized reservoir pond compared to the creeks, the culture ponds and the fertilized reservoir pond because during the first month of the culture period, a high number of fish and other predators were feeding on the available algae in the fertilized reservoir pond. In the culture ponds algae could be filtered by *Artemia* hence their low concentration. Toxic algae species showed up frequently probably due to the presence of some nutrients (though low) in the water column favouring those harmful algal species.

Hoffmann (1999) reported that tropical marine ecosystems are characterized by a unique cyanobacterial flora the main reason being due to the temperature that limits their geographical distribution. Rahman (2006), while working in Indian solar saltworks, reported the presence of cyanophycean filamentous algae such as *Oscillatoria sp*., *Lyngbya sp*. and diatoms such as *Navicula sp*., *Nitzschia sp*., *Pleurosigma sp*. etc., the same species as observed in this study, within a wide range of salinity between 41 – 150 g/L. The presence of these large filamentous algae together with their toxicity could be the cause of *Artemia* mortalities during the 6th and 21st day of the culture period.

In our study, high numbers of *Acartia negligens* and *Harpacticoid sp*. dominated the non-*Artemia* zooplankton in some culture systems at different time. The high number of predators in creek 1 might have resulted from the low salinity in the creeks (35 – 45 g/L). Anh (2009) found the presence of copepods in the culture ponds when the salinity was low (<80 g/L), which also occurred in this study in creek 1 during the second month. However, the salinity in the control and fertilized culture ponds of this study was approximately 100 g/L (Table 4.1), but still

high numbers of *Acartia negligens* and *Harpacticoid sp.* were observed during the first two months of the culture period. Probably, those predators have well adapted to Kenyan environmental conditions allowing them to survive at such high salinity. Toumi et al. (2005) while studying the seasonal distribution of metazooplanktons and large-sized ciliates in 4 ponds with different salinity in Sfax (Tunisia) found that copepod and rotifer abundances are negatively affected by salinity (40 – 90 g/l). The population of the predators reported by these authors was much higher compared to this study.

5.1.4 *Artemia* parameters

Population density and composition

In general, population density showed a gradual decrease at day 14 as compared to the first weeks. A rapid increase in population density was observed from day 21 up to the end of the culture period. The maximal average value reached at day 28 was 950 animals/L in the fertilized culture ponds. Significant differences found were in the fertilized culture ponds having higher densities than the control culture ponds in the last two weeks of the culture period. Significantly higher population density on days 21 and 28 for the fertilized culture ponds than the control could probably be due to release of the second generation of *Artemia* (Figure 4.7). Population density in the control was low compared to fertilized culture ponds possibly due to the presence of high number of copepods (*Harpacticoids sp*.) feeding on the nauplii or competing for the food in the culture ponds. Anh et al. (2009) reported a population density of 80 individuals/L after six weeks of the culture period while Lulijwa (2010) showed high population density (174 individual/L) of *Artemia* in green water supplemented with tapioca and rice bran in week 5, which are lower values than the maximal densities observed in our study.

Maturity

A higher maturation percentage (70%) was found in the fertilized culture ponds than in the control culture ponds (30%). High maturation in ponds with high nitrogen and food availability (as in this case for the fertilized culture ponds) has been reported by several authors. Anh et al. (2009) reported early maturation in the ponds supplemented with nutrients or with increasing food availability. Wurtsbaugh and Gliwicz (2001) and Lulijwa (2010) observed high growth and percentage maturation when supplementing *Artemia* feeds with chicken manure and pig manure. According to Lulijwa (2010) the type of food has an important role in speeding up the maturation rate of *Artemia* and inert diets with high carbon content tend to initiate the maturation as it was found by green water supplemented with tapioca and rice bran treatment in his work. In our fertilized treatment riding couples were seen on the 10^{th} day, nauplii observed on the 15^{th} day while cysts were observed on 16^{th} day. In the control ponds coupling was observed on the 10^{th} day, nauplii observed on 18^{th} day and cysts were not observed before 28^{th} day of the culture period.

Dry weight and total length

Dry weight and total body length increased from day 7 to 28. Generally animals in the fertilized culture ponds had a significantly higher ($p<0.05$) body weight than those in the control culture ponds at the very end of the culture period. Also, the animals showed significantly longer total length in the fertilized culture ponds than in the control culture ponds from day 14 onwards. The poor increase in total length in the control run (up to 4.8 mm at the end of the culture period) could be caused by the presence of blue-green algae and insufficient amounts of

beneficial algae, whereas probably good quality diatoms for the *Artemia* could have played an important role in the fertilized culture ponds. Baert et al. (1996) found that not all algae are consumed by *Artemia* but green algae such as *Tetraselmis sp*. and *Dunalliela sp.,* and diatoms such as *Nitzschia sp*., *Navicula sp*. and Cha*etoceros sp*. are most suitable for *Artemia*.

Most of these species were observed in the fertilized reservoir pond and fertilized culture ponds due to the application of fertilizer and manure that boosted the phytoplankton growth. Lulijwa (2010) reported the highest total length (in the range 8-10 mm) in the treatment with green water supplemented with tapioca and chicken manure which is more than the maximum length observed in our study (7.2 mm in the fertilized culture ponds). Johnson (1980) and Teresita and Leticia (2005) proposed that carbohydrates in inert diets like rice bran contribute to the *Artemia* growth as they need plentiful carbohydrate at the first days of the development. Apart from food insufficiency, high temperature fluctuations could also have been the reason for the retardation of *Artemia* growth.

Fecundity

Both oviparous and ovoviviparous modes of reproduction were observed dominating in the control and the fertilized culture ponds at different times. Fecundity of oviparous females increased rapidly from week 2 to 3 and then dropped in week 4 in the fertilized culture ponds. In the control culture ponds, fecundity of oviparous female was low in week 1 but gradually increased in week 3 and remained stable in week 4. In general, fecundity of oviparous females was significantly ($p<0.05$) higher in the fertilized culture ponds than in the control in weeks 3 and 4 with a maximum of 37 cysts/brood observed in week 3 in the fertilized culture ponds. Also, ovoviviparous fecundity was higher in the fertilized culture ponds than in the control. In this mode of reproduction a maximal value of 40 nauplii per brood was observed in week 3 in the fertilized culture ponds.

Literatures data show that several factors are known for initiating switching of reproductive modes. D'Agostino (1965) and D'Agostino and Provasoli (1968) reported that food quality and/or amount of food (low quality and/or quantity) tend to stimulate oviparity, and Vos and de La Rosa (1980) reported that food type and the female's reproductive profile or history can determine the mode of reproduction in *Artemia*. Also, Sorgeloos et al. (1976) elucidated that oxygen levels below 2 mg/L stimulate the formation of haemoglobin that in turn leads to shift from ovoviviparity to oviparity. Also other studies demonstrate harsh conditions in general stimulate oviparity (Pinto Perez., 1993; Triantaphyllidis et al., 1995; Van Stappen, 1996; Clegg and Gajardo, 2009).

Artemia cyst yield

Cyst yield went down from week 4 (61.10 gDW) to the end of the culture period in the fertilized culture ponds, while it was minimal in the control ponds throughout the culture period, with some yield observed only in week 5 and 6. Relative higher cyst yields in the fertilized culture ponds in week 4 and 5 might be related to the high density of the adult *Artemia*. Lulijwa reported much higher values of cyst yield (17.25 kg ww/ha/week) than this study. In Vietnam about 83 kg ww/ha and 123 kg ww/ha total yield per season in one cycle and multi-cycle respectively can be earned (Baert et al., 2008). High cyst yield may be a result of manure and fertilizer application in the fertilized reservoir pond. Schroeder (1980) explained that poultry manure is a superb substrate for the growth of microorganisms, especially bacteria, associated

with the organic matter, boosting the development of high population of bacteria which is the adequate food for *Artemia* resulting into high cyst production. Arredondo (1993) reported for the presence of high nitrogen and phosphorus elements in the poultry manure that are favourable nutrients for the growth of beneficial algae. The fertilized culture ponds and fertilized reservoir pond were found to have suitable algae for the *Artemia* such as diatoms and green algae that played a big role in boosting cyst production. Low cyst yields in the control are probably the result of unfavourable and low food together with low population density of *Artemia*.

5.2 Laboratory experiments

5.2.1 Effect of temperature on survival, growth and reproduction

5.2.1.1 Survival

The results showed that survival decreased as the temperature increased. A higher percentage of animals surviving was found before day 12 at 28°C than at 32°C. At 36°C drastic mortalities were observed from day 0 up to the end of the culture period. These results are in agreement with the observation reported by Hoa when culturing *Artemia* at 38°C. This was explained by Baker (1966) and Vanhaecke et al. (1984) that, higher temperatures (above 35°C) are lethal to *Artemia franciscana*. However Kn2 showed higher survival (21%) compared to the other strains suggesting that they are well adapted to high temperatures. Generally VC, Kn2 and SFB indicated higher survival at 28°C than at 32°C implying that the optimal temperature condition for their survival is around 28°C.

Two way ANOVA analysis of the survival showed a significant interaction between the strain and the temperature in this study after day 12 up to the end of the culture period. This implies that during this period strains responded differently with the increase in temperature.

Anh (2000) explained that the ability of the strains to tolerate a certain temperature range refers to the natural environment although Vanhaecke et al. (1984) recommended that for all strains the common optimum range of temperature is between 20 to 25°C. Since Kenyan and VC strains are from the same origin (San Francisco Bay) then they are adapted to their habitat as well. Vanhaecke et al. (1984) reported that the maximum temperature for survival of SFB is 20.6°C, which is lower than the temperature range (28 - 36°C).

Furthermore, according to Vanhaecke et al. (1984), *Artemia franciscana* strains clearly show differences in temperature tolerance within the same sibling species. This is a common phenomenon since a noticeable degree of genetic differentiation may take place among strains belonging to the same sibling species (Abreu-Grobois and Beardmore, 1980 and 1982). Since Kn1, Kn2 and VC strains originated from the strain living in San Francisco Bay where the temperature rarely exceeds 24°C, but have been inoculated in saltworks with higher water temperatures (Vos et al., 1984) the increased temperature tolerance of these strains confirm a heritable adaptation to high temperature conditions. In southeast Vietnam the maximum air temperature is 33°C (water temperature >40°C) (Hoa et al., 1994 and Hoa 2003) while in Kenya the maximum air temperature is 36°C (Africanguide, 2011) (water temperature rises up to 42°C, personal observation). The capacity of SFB *Artemia* to stand high temperatures (Browne and Waganisera, 2000) has helped the species to continue colonize new habitats, such as in Kenya and Vietnam.

5.2.1.2 Length

In our study, from day 4 to 8 of the culture period all strains grew fast at both temperatures, growth was retarded in between day 8 and 24, followed by a faster growth again from day 16 to 24 at 28°C. At 32°C, slow growth was observed over a long time (from day 8 to 20), but then the animals showed an increased growth from day 20 to day 24. The animals cultured at 32°C showed somewhat higher length at day 24 (11.8 cm, VC strain) than at 28°C (11.1 mm) (Table 4.10).

At the end of the culture period VC showed the highest length (11.1 and 11.8 mm) at both temperatures. Also, Kn2 strain performed better (in terms of length) at 32°C than at 28°C. The results of this study are in agreement with the work of Vanhaecke and Sorgeloos (1980b) and Anh (2000) that growth is a strain specific characteristic. Anh (2000) reported that the difference in growth rate might be the result of genetic adaptation to the environmental temperature. Likewise, Reeve (1963) and Kinne (1970) reported that the growth rate of the organisms tends to increase with the increase in temperature. Vanhaecke and Sorgeloos (1989), and Hoa (2000) reported that temperature has an effect on the growth performance of *Artemia*. Therefore difference in growth rate between the test strains at 28, 32 and 36°C may be due to genetic adaptation to the environmental temperature (Anh 2000).

5.2.1.3 Reproductive characteristics

In this study, all reproductive parameters analyzed with the exception of percentage offspring encysted, total broods per female, oviparous and ovoviviparous brood per female and offspring per female per day indicated a significant interaction between the strain and temperature. This means that different strains react differently with the increase in temperature and if the temperature is changed it will affect one strain or another in a different way. Several authors (Von Hentig, 1971; Browne et al., 1988; Wear and Haslett, 1986b; Sanggontanagit, 1993 and Anh, 2000) reported that the change in temperature affects the life-history and reproductive traits of *Artemia*. Usually females with a short reproductive period produce a small number of large broods with a very short time between broods while those with longer reproductive period tend to distribute their reproductive effort (Lenz, 1987; Anh, 2000).

At 28°C the highest number of total offspring per female was recorded for the VC strain (210 individuals/female; the value was significantly higher than for the Kenyan strains) while at 32°C Kn2 strain had high number of offspring per female (67.8 individuals/female). Generally all strains (with the exception of Kn2) cultured at 28°C produced higher total offspring compared to 32°C. This shows that Kn2 had a better response to high temperatures than the other strains. The adaptability of Kn2 to high temperature condition is supported by the findings of Vos et al. (1984) who reported that, temperature tolerance in *Artemia* strains originated at San Francisco Bay and then cultured in other geographical environments with pronounced higher average water temperatures tends to produce better output than the parental material. Offspring per female per day was high for all strains at 28°C than 32°C in our work. The Kenyan strains had more or less the same number of offspring per female with VC at both temperatures suggesting that they are better adapted to high temperature condition. Our results were in agreement with the results of Anh (2000) who found lower values with females (*A. franciscana*) having higher total offspring production in most strains and against the findings of Browne et al. (1988) who reported higher values with females having higher total offspring production in most populations. Also, the findings of this study are in agreement with the observations of

Sanggontanagit (1993) who stated that *Artemia franciscana* shows lower number of offspring per brood with the increase in temperature.

Anh (2000) and Browne et al. (1984) reported that the total reproductive productivity imitates the combination of other reproductive traits. The results of this study showed that total offspring per female for most of the strains went hand in hand with the reproductive period; the same trend of observation was reported by Anh (2000). It was observed that VC and Kenyan strains showed similar or different response and sometimes Kenyan strains showed even better response than VC strain when exposed at different or the same temperatures. For example, VC indicated a longer (12.6 days) reproductive period than Kn2 at 28°C while Kn2 showed the longest reproductive period at 32°C. Anh (2000) stated that the reproductive period is one of the important parameters reflecting the animals' resistance to high temperature that was revealed in our study. As the temperature was increased the reproductive performance was reduced for all strains with the exception of Kn2. Probably, Kn2 that lived in Malindi for almost 15 years is more adapted to Kenyan high temperature condition even better than Kn1 that lived there for 2 decades! Likewise, in this study VC strain showed high total number of broods (2.9) at 28°C while Kn1 dominated at 32°C.

Furthermore, offspring per female per day was high in all strains at 28°C than 32°C in our work. It was indicated that Kenyan strains had more or less the same number of offspring per female with VC at both temperatures suggesting that they are better adapted to high temperature condition. The findings of this study are in agreement with the observations of Sanggontanagit (1993) who stated that *Artemia franciscana* shows lower number of offspring per brood with the increase in temperature. The highest percentage of encysted offspring was shown by Kn2 and SFB (11%) at 28°C while at 32°C Kn1 had the highest value (75%). All strains exposed at 32°C showed high values than at 28°C (with the exception of SFB). This observation is against the findings of Anh (2000) who found that at high temperature (30°C) *A. franciscana* strains reproduced significantly lower percentage encysted offspring than at low temperature (26°C). At both temperatures the lowest brood interval found was 1.1 days (SFB) and the longest was 2.4 days (Kn2). In this study lower brood interval were observed compared to the work of Anh (2000) who observed the brood interval of between 2.2 to 3.1 days. The cause of this phenomenon was stated to be the metabolism of the animal as for *Artemia* (poikilothermic organism) the metabolic rate depends on the body temperature (Sanggontanagit, 1993).

5.2.2 Heat shock experiment

Survival in the induced thermotolerance test was Kn2>VC>Kn1>SFB, which was not expected because the K1 strain (cysts collected in 2010) had been living in Malindi conditions for two decades, whereas Kn2 has lived there for almost 15 years. No significant differences were detected between VC and Kenyan strains. Probably all Kenyan strains and the VC strains are adapted to the high temperature in their habitat compared to SFB strain which is known for its adaptation to low temperatures (maximum 24°C).

The same findings were reported by Clegg et al. (2000) who stated that adults of the second generation (adults from the cyst of *Artemia franciscana* originated from San Francisco Bay and inoculated in Vietnam) showed higher heat resistance than the original SFB strain when exposed at 38°C for 1 h and survival recorded after 3 days. The results in mean survival of VC in our study (65±12%) recorded after 23 h may not be entirely comparable with the results of

Clegg et al. (2000) as these authors reported survival (48.6±5.5% for VC) after 3 days of heat shock.

5.3 Socio-economic study

According to Ochiewo (2004) fishing is traditionally a male activity in East Africa but due to the rising economic problems women are nowadays involving in fishing activity. *Artemia* culture activity (especially pond culture) is not restricted to one sex only but all people above 18 years of age can be involved. Fishing is the main economic activity but due to lowered income generated by capture fisheries caused by the use of inappropriate fishing technology (Ochiewo, 2004), *Artemia* culture may be the solution for this problem to boost aquaculture sector and hence food security.

Since the people who responded to the questionnaires were either working at the pond site, artisanal saltworks, salt industries or benefited in one way or another from the presence of the ongoing project they heartily appreciated the project. A very high percentage of the people was aware of the project and of *Artemia* culture in general because a number of women and men were hired in the actual stage of pond construction. Also some of the elders witnessed the importance of *Artemia* during the year 1980s when Kenyan saltworks industries at Magarini district were invaded with algal blooms. During that time, *Artemia* was introduced for the first time in Kenya and helped to solve this problem by clearing the ponds affected with algae blooms (Nyonje, personal communication; Bossier et al., 2010). A large group of people had at least a basic education, therefore these people can be trained in the proper way of culturing *Artemia* and spread this new technology when the project reaches a more advanced stage.

6 CONCLUSIONS AND FUTURE RESEARCH

6.1 Conclusions

6.1.1 Field work

The fertilization programme with both inorganic (Urea and DAP) and organic manure (poultry manure) led to significantly higher ($p<0.05$) nutrient levels parameters in the fertilized treatment and fertilized reservoir pond, especially during the third month, as compared to the control. This promoted the growth of favourable algae such as diatoms and green algae that were consumed by *Artemia*, as illustrated by the better growth, higher density, earlier maturation and higher fecundity in the fertilized culture ponds.

Generally, *Artemia* culture in Kenya is a new technology and it is still on its initial stage. Nevertheless, the *Artemia* culture project was appreciated by the Kadzuhoni community, that was eager to learn and interested to see better results. It may be expected that once the project reaches a more advanced stage and the technology spreads to the nearby communities, more people will get more employment opportunities through *Artemia* pond culture.

Considering the results and observations of this study, some technical problems still need to be settled down. Apart from providing constant high saline water, proper routine and the right amount in fertilizing the ponds with inorganic fertilizer and manure in the ponds, adding supplemental feeds will improve *Artemia* production in Malindi. It was observed that one of the neighbours (artisanal farmer) at the experimental site in Malindi was frequently blocking the creek resulting into shortage of water into the culture system. To avoid this unnecessary problem it's better to install the culvert so that the artisanal farmer can transport his salt and the *Artemia* project farm can get enough water. Once the problem is solved more production in the ponds is thought to be attained. Also, it is better to construct a project house so that the documents and equipments can be stored safely and this will go hand in hand with the improvement of infrastructure at the farm**.**

6.1.2 Laboratory experiments

The different strains of *Artemia* analyzed (Kn1, Kn2, VC and SFB) reacted differently at 28, 32 and 36°C with respect of survival and growth, which may be explained by adaptation to the environmental condition.

SFB strain survived well at 28°C compared to other strains while Kn2 performed even better than VC at 32°C. In spite of massive overall mortality, Kenyan strains seemed to perform better at 36°C than VC and SFB. Most spots reliable for *Artemia* inoculation are situated in the sub-tropical and tropical areas where water temperatures may exceed even 30°C. The victory of inoculation tests is really dependent on the selection of the strains with good qualities such as tolerance in high temperature and good production performance at high temperatures; in this regard, Kn2 should be given the first priority followed by VC and Kn1.

Generally, most of the reproductive characteristics revealed that Kenyan strains had a better reproductive performance at high temperatures compared to the other strains. High percentage of encysted offspring, high number of total offspring per female, high total number of broods and the longest reproductive period were observed at 32°C. This implies that Kenyan strains (especially Kn2) are well adapted to high temperature condition. Due to this better

response of Kenyan strains and VC strain they may be regarded as the most superb strains for introduction into tropical and sub-tropical areas.

This relatively good performance of the Kenyan strains at higher temperature was confirmed by the heat shock test, which demonstrated the optimal performance of the Kn2 strain after 3 consecutive heat shocks. With increasing in technology in Kenya, this strain may be considered as a promising resource that is likely to perform even better than the VC strain from Vietnam.

6.2 Future research

In order to have a better understanding of the success of *Artemia* pond production in Malindi, Kenya, it is important to have more accurate knowledge on the effect of feed supplementation in the culture ponds. Supplementation of feed using agricultural and industrial food processing by-products, together with other fertilizing agents such as manure or inorganic fertilizer have been documented to improve the production in the culture ponds.

Future studies aimed at promoting the development of suitable or beneficial algae and bacteria as important food for *Artemia* are therefore suggested. As found in the field test, fertilizing the pond is of utmost important and therefore feeding strategies attempting to improve the cyst yield production, maturation and biomass production need to be supported.

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APPENDIX

Integrated *Artemia* production in a mangrove wetland at the Kenya coast ID NO. ……

QUESTIONNAIRE

DATE …….. VILLAGE…….. NAME OF THE INTERVIEWER…… ……

NAME OF THE INTERVIEWEE……AGE OF INTERVIEWEE (in years)…….. SEX……

POSITION OF INTERVIEWEE IN THE HOUSEHOLD……..

PART I: HOUSEHOLD CHARACTERISTICS

1 **NUMBER OF HOUSEHOLD MEMBERS** *(Make a complete list of all individuals who normally live and eat together in the household. START WITH THE HEAD OF THE HOUSEHOLD)*	**2** **SEX OF EACH HOUSEHOLD MEMBER** ***Male=1*** ***Female=2***	3 **AGE OF EACH HOUSEHOLD MEMBER**	4 **RELATION OF EACH HOUSEHOLD MEMBER** **TO H/H HEAD** ***Head=1*** ***Spouse=2*** ***Son/Daughter=3*** ***Father/Mother=4*** ***Sister/Brother=5*** ***Grand child=6***	**5** **MAIN OCCUPATION OF EACH HOUSEHOLD MEMBER** ***Farmer=1*** ***Fisherman=2*** ***Business/trader=3*** ***Employee=4*** ***Seeking job=5*** ***Sick/incapacitated=6***	**6** **EDUCATION** What is the highest level of education attained by each household member? ***None=0*** ***Incomplete primary=1*** ***Complete primary=2*** ***Complete primary+ a course=3*** ***Incomplete secondary=4***	**7** **ORIGINAL DOMICILE** *Where was the head of your household born?* ***Within this village=1*** ***Another village in the same district=2*** ***Another coastal distric*** ***Another non coastal district within Kenya=4*** ***outside of Kenya=5***	**8** **CURRENT DOMICILE** *How long has the head of household lived in this village?* ***Since birth=1*** ***10 or more years=2*** ***1-10***	**9 RELIGION** ***Muslim=1*** ***Christian=2*** ***None=3*** ***Other (Specify)=4***

			Other relative=7 *Live in servant=8* *Other non relative=9*	*Retired/too old=7* *Full time student/pupil=8* *Housewife=9* *Other (specify)=10*	*Complete secondary=5* *Complete secondary + a certificate course=6* *Diploma training=7* *University=8* *Madrasa=9* *Other (specify)=10*		*years=3* *0-1 year=4*	

PART II: INCOME & LIVELIHOOD

1) How much income do you earn from the main occupation (income activity) in *Kshs. p*er day/week/month?

1–3,000	3,001–10,000	10,001–20,000	20,001–30,000	30,001 and above

2) From which other activities do you earn income? ……
……
……
……

3) Income from other activities in *Kshs.*

1–3,000	3,001–10,000	10,001–20,000	20,001–30,000	30,001 and above

4) If married, what is the occupation of your spouse? ……..

5) What do your children do? …….

6) Income earned by spouse?

1–3,000	3,001–10,000	10,001–20,000	20,001–30,000	30,001 and above

7) How many members of your household are working? ……
……
……

8) Income earned by other members of the household in *Kshs*

1–3,000	3,001–10,000	10,001–20,000	20,001–30,000	30,001 and above

9) How much money do you use per day? ……

Please rank how you use your income:

Item	Rank	Item	Rank
Food		Leisure	
Education		Bank/savings	
Clothing		Investment (specify)	
Health/medical care		Other (specify)	
Shelter			

PART III. HOUSING CHARACTERISTICS

10) How many houses are owned by the family in the same land? .....

Of what material is the house(s) constructed?

Houses in the same land	Type of roofing 1= Thatch grass/coconut leaves 2= iron sheets 3=Tiles 4=Concrete/cement 5=Other	Type of wall 1=Thatch grass/coconut leaves 2=Clay 3=Stone 4= Other? Specify	Number of rooms
House 1			
House 2			
House 3			
House 4			
House 5			

PART IV. ASSETS OWNED

Land		**Size in acres**	
Total land owned by the household			
Size of land devoted to food crops			
Size of land devoted to cash crops			
Size of land devoted to livestock grazing			
Size of land used by others			
Asset	**No.**	**Asset**	**No.**
Cattle		Bicycle	
Goats		Motor cycle	
Sheep		Vehicle	
Chicken & ducks		Sofa set/chair	
Pigs		Bed	
Donkeys		TV	
Fishing gears (specify)		Radio	
Boat (specify type)		Other (Specify)	

11) Do you own any of the following assets?

Asset	**No.**	**Asset**	**No.**
Cattle		Bicycle	
Goats		Motor cycle	
Sheep		Vehicle	
Chicken & ducks		Sofa set/chair	
Pigs		Bed	
Donkeys		TV	
Fishing gears (specify)		Radio	
Boat (specify type)			

PART V: *ARTEMIA* CULTURE

12) Have you heard of *Artemia*?

13) What is it used for?

14) When did you first learn of artemia culture?

15) Is *Artemia* culture valuable to you?

16) How?

17) If involved in the *Artemia* culture project, how have you benefited?

……
……
……

18) What should be done to make it benefit more people in your community?

19) In your opinion, who among the community members should be targeted by the *Artemia* project?

20) What challenges do you experience?

PART VI: FOOD SECURITY AND COMMUNITY WELFARE

1. How many meals does your household usually have per day?

   Number ☐

2. In the past 30 days has your household ever had fewer meals than this usual number?

   Yes…… = 1 ☐ If No go to Q.12.6

   No……. = 2

3. If Yes, how many days? Number ☐

4. How often in the last year did you have problems of satisfying the food needs of the household?

   Never…… = 1

   Seldom……. = 2 ☐

   Sometimes…… = 3

   Often…… = 4

   Always……. = 5

5. How do you compare the overall economic situation of the HOUSEHOLD with one year ago?

   Much worse now……. = 1

A little worse now…… = 2

Same…… = 3

A little better now…… = 4

Much better now…… = 5

Don't know…… = 6

6. How do you compare the overall economic situation of the COMMUNITY with one year ago?

Much worse now…… = 1

A little worse now…… = 2

Same…… = 3

A little better now…… = 4

Much better now…… = 5

Don't know…… = 6

7. How does this household compare with the others in this COMMUNITY?

Much worse now…… = 1

A little worse now…… = 2

Same…… = 3

A little better now…… = 4

Much better now…… = 5

Don't know…… …. = 6