APPROVAL SHEET

This research paper entitled "PERCENT SURVIVAL OF THREE- WEEK OLD NATIVE CATFISH (*Clarias macrocephalus* Gunther) FRY UNDER SIMULATED TRANSPORT CONDITIONS AT DIFFERENT LOADING DENSITIES AND TRANSPORT DURATION" prepared and submitted by **Julie Ross B. Amor and Kathleen Joy B. Taleon** in partial fulfillment of the requirements in Science Research II, has been examined and is recommended for acceptance and approval.

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PERCENT SURVIVAL OF THREE- WEEK OLD NATIVE CATFISH (*Clarias macrocephalus* Gunther) FRY UNDER SIMULATED TRANSPORT CONDITIONS AT DIFFERENT LOADING DENSITIES AND TRANSPORT DURATION

A Research paper
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Faculty of Philippine Science High School
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PERCENT SURVIVAL OF THREE- WEEK OLD NATIVE CATFISH (*Clarias macrocephalus* Gunther) FRY UNDER SIMULATED TRANSPORT CONDITIONS AT DIFFERENT LOADING DENSITIES AND TRANSPORT DURATION

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ABSTRACT

This study determined the effect of different loading densities and duration of transport on percent survival of three-week old native catfish, *Clarias macrocephalus* fry within two weeks after simulated transport. Three-week old native catfish fry were collected from the larval rearing tanks (LRT) at the Southeast Asian Fisheries Development Center (SEAFDEC) in Tigbauan, Iloilo.

The native catfish fry were randomly selected and packed at loading densities of 1 000, 2 000 and 3 000 individuals per 10 li in 5 x 8" polyethylene bags containing 100 ml of aerated freshwater (1:2, water: oxygen ratio). Each density level had three replicates. The bags were sealed and placed in a tray secured on top of a laboratory orbit shaker set at 50 rpm for four hours to simulate transport. Another set of fry was subjected to six hours of simulated transport. Percent survival of fry within two weeks after simulated transport were analyzed using Analysis of Variance and Duncan's Multiple Range Test at $\propto = 0.05$ (SAS System).

Results showed that stocking density and duration of transport significantly affected the percent survival of the *C. macrocephalus* fry during transport, and within the two-week culture period. Fourteen days after simulated transport, percent survival dropped (54- 41%) in fry subjected to four hours of simulated transport in all stocking densities. It was significantly lower (26-10%) for those subjected to six hours of simulated transport and was lowest in the group with stocking density of 3 000 fry / 10 li water subjected to six hours of simulated transport.

Results indicate that loading density and duration of transport influence the viability of the *C. macrocephalus* fry. Optimum loading densities resulting in high survival rates can be 1 000 up to 2 000 ind. / 10 li. However, the duration of transport must not exceed four hours. Loading density during transport of *C. macrocephalus* fry must be less than 1 000 ind. / 10 li if the duration of transport will be more than four hours.

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CHAPTER 1:

INTRODUCTION

A. Rationale of the Study

The catfish *Clarias macrocephalus* Gunther is preferred among *Clarias* species because of its tender and more delicious meat. It is a native catfish species but fast disappearing in the Philippines (Fermin, 1998). They were previously observed in ponds, farms and irrigation (Fermin et al., 1995). Many people believed that the disappearance of the native catfish is due to the interbreeding with Thai catfish (*Clarias batrachus*) and the rampant use of pesticides in the rice fields which "poisoned" the natural breeding grounds of this species (Fermin, 1998). In order for their species to flourish, it is usually cultured. Fry and fingerlings are then transported to grow- out ponds, while the larger catfishes are brought to market to be sold.

Transportation is part of the routine fish culture processes (Garcia and Toledo, 1988; Berka, 1986). During and after transport, losses are inevitable. To minimize mortality and loss during conveyance of live fish, several precautions must be taken to improve transport conditions (Pin, 1986). The researchers conducted this study to determine the optimum transport density that will lessen the mortality of the native catfish (*C. macrocephalus* Gunther) fry during and after transport.

B. Statement of the Problem

This research is designed to answer the following questions:

1. Will the different loading densities affect the percent survival of three- week old native catfish (*C. macrocephalus* Gunther) fry during and after simulated transport conditions within the two- week culture period?

2. Will the different duration of simulated transport influence the percent survival of three- week old native catfish (*C. macrocephalus* Gunther) fry during and after transport within the two- week culture period?

3. Which loading density and transport duration will result to optimum survival of the native catfish fry (*C. macrocephalus*) within the two- week culture period?

C. Hypothesis of the Study

There is no significant difference in the percent survival of three- week old native catfish (*C. macrocephalus* Gunther) fry during and after simulated transport conditions at different loading densities and duration of transport within the two- week culture period.

D. Objectives of the Study

1. To determine the effects of different loading densities on percent survival of three- week old native catfish (*C. macrocephalus* Gunther) fry during and after simulated transport conditions within the two-week culture period.

2. To determine the influence of different duration of simulated transport on percent survival of three- week old native catfish (*C. macrocephalus* Gunther) fry during and after transport within two-week culture period.

3. To determine which loading density and transport duration will result to optimum survival of the native catfish fry (*C. macrocephalus*) within the two- week culture period.

E. Significance of the Study

Part of the routine activity of the aquaculture is the transportation of live fish (Quinitio et al., 1991). In fish farming, conveyance of live fish from the hatchery to the grow-outs or the markets is of great importance in the economy. Frequently, live fishes reach their destination in poor state of physiological conditions because of transportation stress, resulting in high mortality during the time of "planting," or during grow- out culture (Mohamed and Devaraj, 1997).

The native catfish, *Clarias macrocephalus* Gunther is a fast disappearing species of catfish in the Philippines. Though preferred for its tender and more delicious meat, this native catfish is now becoming a scarce commodity. Many people believe that the disappearance of the native catfish is due to its interbreeding with the Thai catfish (*Clarias batrachus)* and the rampant use of pesticides in the rice fields that poisoned the natural breeding grounds of this species (Fermin 1998).

The objective of this study is to transport native catfish fry at a loading density and transport rate in which as many as possible will arrive at the grow- out ponds in good condition. The success of aquaculture depends partially on developing some techniques to lessen mortality of fry during and after transport. Furthermore, this study attempts to increase the true- breed population of the native catfish, *C. macrocephalus* Gunther, through aquaculture.

F. Scope and Limitations of the Study

In this study, the researchers used three- week old native catfish (*Clarias macrocephalus* Gunther) fry as their test organisms that were packed in 5x8" polyethylene plastic bags. The loading densities that were used were only 3 000, 2 000 and 1 000 ind / 10 li. For the duration, the researchers made used of four and six hours of simulated transport since it usually takes this long to convey the native catfish fry from the hatchery to the ponds of buyers or of the collaborating agency. The researchers used 18 sampling bottles for every run. This experiment which consisted of two runs per transport duration was conducted for three weeks at the Southeast Asian Fisheries Development Center / Aquaculture Department at Tigbauan, Iloilo.

G. Definition of terms:

***Clarias macrocephalus* Gunther fry** – three-week old native catfish that served as test organisms subjected to simulated transport conditions

Percent survival – number of live fishes per set-up over the total number of test organisms multiplied by 100

Loading density – number of fishes placed per volume of water; used as one of the factors to determine the percent survival of three- week old native catfish after simulated transport

-- the experiment utilized 1 000, 2 000 and 3 000 fry / 10 li as loading densities

Transport duration – the time it takes to convey the native catfish from one location to another; used also as one factor for percent survival determination of the test organisms

-- the conducted study used four and six hours as transport duration of simulated transport

Culture Period – 14 days of observation of the percent survival of the test organisms

Polyethylene Bags – double- lined bags produced from a translucent polyethylene foil that were used as transport units of the native catfishes on simulated transport

Laboratory orbit shaker – a device set at 50 rpm that was used in the study to simulate transport conditions

Larval Rearing Tank (LRT) – a container where native catfish were nurtured before the researchers used them as test organisms

Dependent Variable: The dependent variable determined in this study was the percent survival of the three- week old native catfish fry.

Independent Variable: The study determined whether the two independent variables, namely A) loading density and B) transport duration affect the percent survival of three- week old native catfish fry subjected to simulated transport.

CHAPTER 2:

REVIEW OF RELATED LITERATURE

A. Catfishes

Catfishes are distinguished by the presence of barbels or "whiskers," the lack of true scales, strong spines at the front of the dorsal and pectoral fins. The body is usually partially or completely armored. They have small eyes and therefore rely on taste, smell, and hearing. Many are inactive during the day, coming out to feed at night. Freshwater catfish usually spent much of their time (and lay their eggs) in hollow logs, undercut banks, and other living places. If these are removed, catfish population declines (Grolier Int'l encyclopedia, 1990).

The *Clarias* species have special "talents." They have accessory organs for air-breathing and can survive under adverse conditions even in dry pools. They thrive in all kinds of freshwater habitats such as marshes, rice fields, swamps, etc. They are tolerant to crowding and can be raised at extremely high stocking rates on artificial feeds. Catfishes have high export potentials because these are considered as delicacies in Asian and other countries (Aqua Farm News, 1993).

B. Catfish *Clarias macrocephalus* Gunther

The Asian catfish, *C. macrocephalus* Gunther, is one of the species of catfishes in the family Claridae. The local name in the Philippines is hito and in Thailand, Pla Duk. This species has the ability to survive in almost all kinds of water and can be cultured even in small areas. *C. macrocephalus* Gunther is preferred over *C. batrachus* because of its delicious taste and higher price in the market (Sansrimahachai, 1977). Studies revealed that *C. macrocephalus* is slow growing (Areerat, 1987). This could be one reason why other *Clarias* species have become more popular for culture than *C. macrocephalus.* However, the delicate taste and fine texture of the flesh still make the native catfish a sought-after fish (Santiago and Gonzal, 1996). It is a highly esteemed indigenous food fish in the Philippines, Malaysia (Mollah and Tan, 19831; Ali, 1993) and Thailand (Areerat, 1987). The morphology of this species is similar to that of *C. batrachus,* with grayish- black colour on the body. There are ten vertical strips of white spots on the abdomen. The occipital process of the head has a round lobe. It is harder to find in the natural habitat than *C. batrachus.* (Fish Culture in Integrated Fish Farming, 1998).

C. Artificial Breeding by Stripping

C. macrocephalus has not been observed to spawn by themselves in captivity. Hence, it was necessary to induce them to spawn by injection of different hormones, and manually stripping the eggs after several hours. Another method of induced breeding is hormone injection on both male and female and letting them spawn naturally. The usual method used is induced breeding by injecting hormones and stripping (Hara, 1977).

C1. Selection of Breeders

The presence of a genital pore that is round in the female catfish distinguished it from the male catfish that had a pointed genital papilla.

Good female breeders could be distinguished by good body size (more than 25 cm. In total length), a palpable, soft, and prominent distention of the belly, pinkish to reddish genital port, prominence of blood vessels in the belly region, and bulging side of the abdomen (Hara, 1972; Carreon, 1973). Differently, male catfish was slimmer than the female's. the papilla of the males should be reddish in color (Hara, 1972).

Due to handling, breeders acquired from markets become weak and therefore needs conditioning before used for induced breeding. This is done to lessen mortality of breeders after hormone injection and also to avoid the waste of hormone. However, it is not necessary to condition breeders raised from their fingerling stage (Hara, 1977).

Stripping must be done when the eggs are totally mature; the abdomen of the breeder must be firm and there must also be a possibility of natural ovulation. Good eggs, more often than not, come out of the genital pore even by a slight touch of the abdomen (Hara, 1972).

C2. Selection of Eggs

The eggs that come out from the breeder is a mixture of good and bad eggs, especially if the amount of injected hormone is excessive. This causes the mature and immature eggs to mix. By induced spawning, the mature eggs kept longer inside the body become over-mature and unfertilized and won't hatch anymore.

Eggs that are yellowish-brown in color are mature eggs. Shortly, after placing in water, its cell division begins and continues to develop. Over-mature eggs cannot be easily distinguished from mature ones. However, after few minutes or hours after placing them in water, they expand, turn white and subsequently die. On the other hand, immature eggs are yellow in color and become white shortly after being placed in water.

In the process of dying, bad eggs break and pollute the water, thus, must be immediately removed to prevent the water from contamination by aquatic germs and also to lessen water pollution (Hara, 1972).

C3. Hatchery and Nursing

After yolk resorption, that is four to five days after hatching, native catfish larvae are stocked at 30 per liter in bigger tanks (Fermin et al, 1995). For thrre days, they are fed with brine shrimp *Artemia* and *Moina* (water flea) for another four days. After that, larvae can be weaned to formulated diets that are given twice a day for two to four week old native catfish fry at 20% body weight (feeding rate) and to older fry at 5-10% body weight (Fermin and Bolivar, 1996).

Ten days before stocking, the nursery tank or pond must be fertilized. Fifteen- day old fry may be stocked at 800 / m^2 and up to 1 200 / m^2 in ponds. Fingerlings can be harvested after 28 days, ready to be stocked in grow- out ponds. More fingerlings can be obtained when fry are grown in net cages suspended in tanks or ponds (Fermin and Bolivar, 1991).

D. Preparation of Fish for Transport

Fishes should be kept in clean water in separate tanks a few days before transport. Weak, injured and diseased fishes have to be taken out and must be replaced. The fish should not be fed for some days, depending on their sizes. Withholding feed allows it to pass out in the digestive tract, reducing the waste buildup that might occur during transport. It has already been established that starvation does not affect the metabolic rates in fish for one week. Holding facility must be provided with continuous aeration through air- diffusers either by compressor or aerator. Size and species sorting and counting take place during pre-transport maintenance (Mohamed and Devaraj, 1997).

E. Causes of Mortality in Transport Operation

According to V. Ramachandran (1969), mortality of live fish at all stages during and immediately after transport may be caused by the following: a) Oxygen starvation of blood and tissues; b) Accumulation of toxins in the transport water; c) Hyperactivity, strain and exhaustion; d) Diseases contracted during transport; and e) Physical injuries caused by jolting, and possibly, by aggressive predatory attempts or accidents due to overcrowding and starvation. These causes may be exclusive or contributing, hence, remedial measures are chiefly coinciding in their effects of course with differing importance.

F. Factors to Consider in Transporting Fishes

Transportation of live fish from one area to the other in aquaculture is unavoidable—from hatcheries to fish farms, fish farms to markets, and other destinations. Oftentimes, large quantity of fry, fingerlings and adult fishes are presently transported. However, to minimize deaths and loss during and after transport, it is necessary to take precautions and consider factors in the transportation.

F1. Oxygen

The most crucial factor to consider in the transportation of live fishes is an adequate supply of dissolved oxygen (DO). A suitable physiological standard for the oxygen consumption rate of fish in transport is half the rate of respiration a fish is capable of beyond the level necessary for subsistence in the resting condition (Pin, 1986).

F2. Water Temperature and pH

Another important factor is the water temperature. The lower the temperature, the higher is the DO level and the lower is the oxygen consumption. Furthermore, lower temperature reduces stress to fish (Pin, 1986). Carbon dioxide and ammonia are more damaging at higher temperatures. Excessive, and particularly sudden, changes in temperature will not allow the body function of a fish to adapt before severe stress and body changes happen (Johnson, 1979). pH of water is also essential. The pH of water between 6.5- 8.5, based on experiments done, is absolute for most fish (Pin, 1986).

F3. Ammonia

The majority of the nitrogenous excretory product of fish is ammonia that accumulates in dangerous amounts when fish are crowded. More ammonia is released when temperature is high. All forms of ammonia are highly toxic substances and its toxicity is highly complex to fish. Ammonia may either be in two basic forms when released into the water: combinable (ionized) ammonia or free (unionized) ammonia. The free form is poisonous to fish. Among its deleterious effects, ammonia decreases the capability of fish to consume oxygen and increases the amount of oxygen necessary (Johnson, 1979).

F4. Carbon Dioxide

As a result of metabolic excretion, fish transported are subjected to increased carbon dioxide concentration. High dissolved oxygen levels inflict stress on the oxygen transport system of fish. CO_2 interaction with ammonia has significant effect. As the CO_2 concentration increases, the pH decreases and the percent of toxic unionized ammonia decreases.

Nevertheless, if the concentration of the unionized ammonia is held steadily, increases in CO_2 level increases the toxicity of ammonia (Mohamed and Devaraj, 1997)

F5. Loading Density

The purpose of the fish culturist is to transport fish at a rate in which as many as possible will arrive in good condition. Loading density in transport varies considerably according to respiration rate of fish, water temperature, transport duration, method of transport, size of fish, etc. Safe loading density is inversely proportional to water temperature. In some cases, a capacity load is not required and the lighter load minimizes the risk damaging effects. On the other hand, duration of transport influences loading density, which is inversely proportional to transport duration (Johnson, 1979; Pin, 1986).

G. Closed System of Fish Transport

Represented by polyethylene bags and other sealed transport units, the closed systems are used mainly for the transport. The transport of fry in polyethylene bags with oxygen is a very efficient method. Thus, it is particularly widely accepted in the world today. It essentially reduces the total content and weight of transport water, making possible for the public transport to be used for fish-transport purposes, likely to lengthen the transport duration, and economically beneficial.

H. Polyethylene Bags

Produced from a thin or thicker translucent polyethylene foil, these bags used for fish transport in water with oxygen atmosphere usually have the shape of sack or sleeve. For security reasons, the bags are sometimes duplicated: a thin bag is enclosed in another thin bag, or a thin bag is outlining the thicker bag.

The polyethylene bags with fry are placed in outer cases during transport to insure them against the mechanical and technical infliction, principally punching or tearing in touching the grounds. The case keeps the bags in the desired position, providing thermal insulation and/ or enables the handling efficiently (Berka, 1986).

I. Stocking

The most critical stage of the transportation can happen in the release of fish at their destination area. The fish are already stressed in the transportation and their sudden exposure to water of diverse or poor quality will further stress the fish, frequently beyond what they can tolerate. Different characteristics of water mean a pH, water temperature or gas saturation difference between the transport unit and the receiving water. The receiving water maybe inappropriate for fish life because of contamination or distinctive natural characteristics. The fishes must have enough strength to adapt to the new environment (Johnson, 1979).

CHAPTER 3:

METHODOLOGY

C1. Materials

The following materials were used in this study:

5x8" polyethylene plastic bags, 72 sampling bottles, 72 aerators, freshwater, plastic bowl, two 100 ml beakers, nine basins, strainer, artificial feeds, thermometer, laboratory orbit shaker, 810 native catfish (*Clarias macrocephalus* Gunther) fry

C2. Experimental Design

This experiment utilized a completely randomized design (CRD). The experimental units are 18 5x8" polyethylene bags containing 100 ml of aerated freshwater with different loading densities of three- week old native catfish fry (*Clarias macrocephalus* Gunther).

The independent variables tested are: loading density and transport duration. Loading density has three levels: 1 000 fry / 10 li, 2 000 fry / 10 li, and 3 000 fry / 10 li. Transport duration has two levels: four hours and six hours. The fry were observed from day one after simulated transport until the 14^{th} day of culture.

The dependent variable observed was percent survival of the native catfish fry. (*C. macrocephalus* Gunther).

C3. Experimental Methods

C.3.1. Induced Spawning of Broodstock

Twenty gravid female native catfish broodstock, approximately 6- 8 months and 30 cm long were induced to spawn by injecting 0.5 µl Ovaprim / g body weight (BW) to hasten ova maturation, and were manually stripped of eggs after 16- 20 hours. Before females were stripped of eggs, male catfish were sacrificed. The male reproductive tract was then dissected, the testes were removed and soaked in 0.6% NaCl for the researchers to easily separate the clotted blood vessels from the testes. It was then macerated to obtain the milt to fertilize the eggs. Thereafter external fertilization, the eggs were placed in flow troughs for observation and hatching.

C.3.2. Larval- Rearing

Four to five days after hatching, native catfish larvae were stocked at 30 per liter in larval-rearing tanks (LRT) and were allowed to grow as fry for three weeks. The water temperature was monitored daily. 70% of water was changed every other day to ensure cleanliness and healthy environment. They were fed twice daily with natural food organisms such as brine shrimp *Artemia nauplii* for three days, and the water flea *Moina macrocopa* for another four days. Thereafter, the catfish larvae were fed with formulated diets with 44% protein and particle size 150- 200 µm. When the fry were three weeks old, they were harvested and used as test organisms for this study.

C.3.3. Simulated Transport Experiments

The experiment utilized a completely randomized design (CRD). Three week old native catfish (*Clarias macrocephalus* Gunther) fry were collected from the larval rearing tanks (LRT) at the hatchery (Plate 1) of the Southeast Asian Fisheries Development Center Aquaculture Department

(SEAFDEC/AQD) in Tigbauan, Iloilo. Initial temperature of freshwater was recorded before the experiment. These test organisms were randomly selected and packed at loading densities of 1 000, 2 000 and 3 000 individuals / 10 li in 5x8" polyethylene plastic bags containing 100 ml freshwater. Plastic bags were then inflated with oxygen (about 1:2, water: oxygen ratio) (Plate 2), sealed tightly (Plate 3) and placed in a tray secured on top of the laboratory orbit shaker and were shaken at 50 rpm for four hours (Plate 4). Each density level has three replicates. A further set of fry was subjected to time duration of six hours of simulated transport. Final temperature of water in each bag was recorded immediately right after shaking. The water and fry from the packs were then transferred into sampling bottles and were aerated for observation.

The survival of native catfish fry was counted one hour after transport and was then recorded. The nitrite and ammonia content of water was measured (Plate 5) a day after simulated transport. Nitrite and ammonia are excretory products of fish that accumulate in dangerous amounts under crowded conditions (Johnson, 1979). Changing of water in each set- up was done every morning. The temperature was also recorded daily (Plate 6). Percent mortality after transport was determined by counting the dead native catfish fry daily for 14 days, during routine maintenance (e.g. cleaning of sampling bottles and feeding of fish in the morning). The percent survival of catfish fry was determined using the formula:

$$\text{Percent Survival} = \frac{\text{no. of test organisms per set-up - dead fry}}{\text{total number of test organisms}} \times 100$$

C.3.4. Statistical Analysis

Two-way Analysis of Variance (ANOVA) at 0.05α (SPSS, Norusis 1986) was used to test whether the factors— A: Loading Density and B: Duration of Transport, have significant effect on percent survival of the native catfish fry within the two- week observation period. When two-way

ANOVA revealed that there is an interaction effect between these factors on percent survival of the fry, each combination of loading density and duration of transport was considered a single treatment (T1 to T6).

The six treatments were subjected to One- Way Analysis of Variance and Duncan's Multiple Range Test (0.05α) (SAS System) to determine which treatment combinations of loading density and transport duration (T1 to T6) varied significantly from each other from day one to day 14.

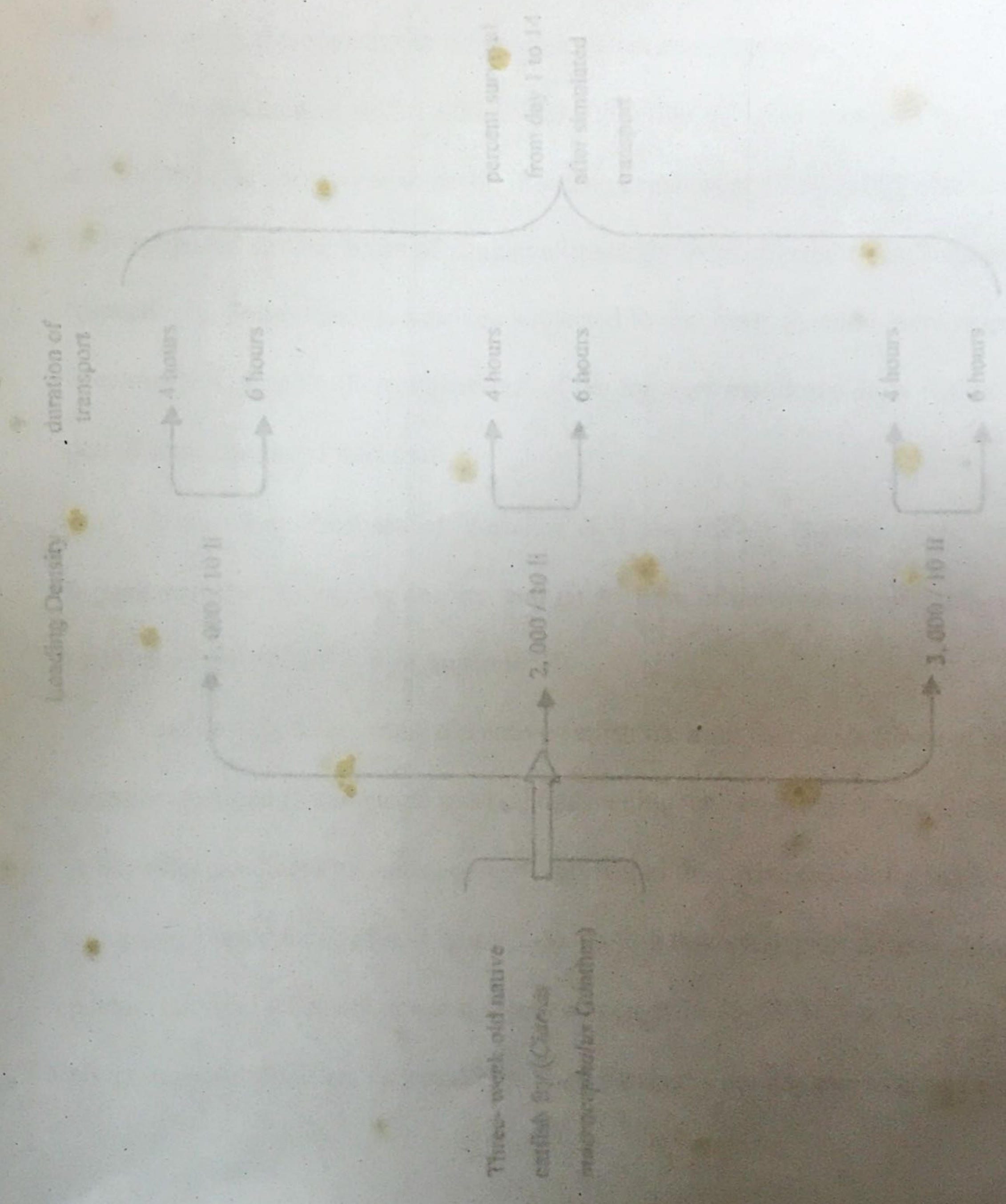


Figure 1: Schematic diagram of

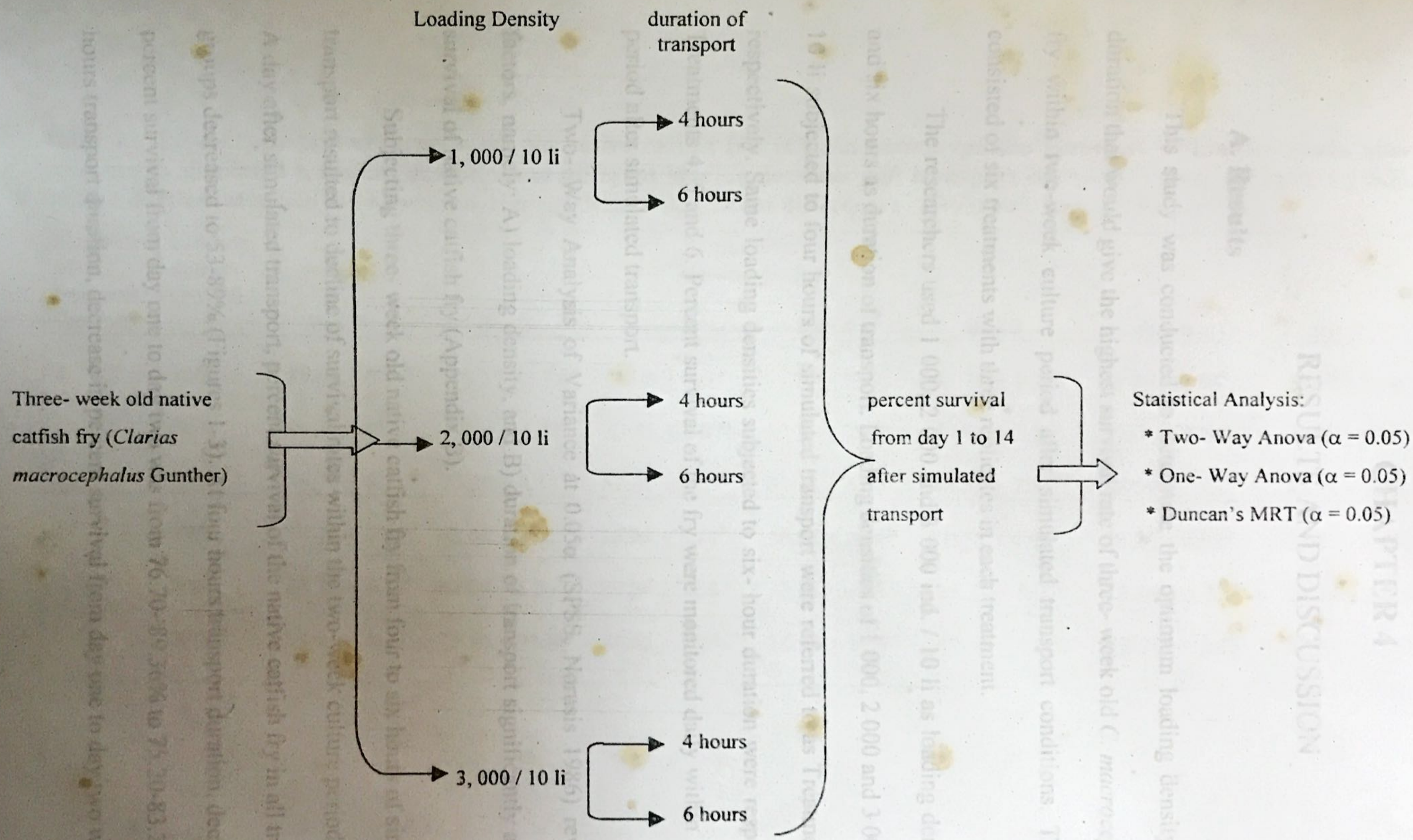


Figure 1: Schematic diagram of the methodology.

CHAPTER 4

RESULTS AND DISCUSSION

A. Results

This study was conducted to determine the optimum loading density and transport duration that would give the highest survival rate of three- week old *C. macrocephalus* Gunther fry within two-week culture period after simulated transport conditions. This experiment consisted of six treatments with three replicates in each treatment.

The researchers used 1 000, 2 000 and 3 000 ind. / 10 li as loading densities and four and six hours as duration of transport. Loading densities of 1 000, 2 000 and 3 000 individuals / 10 li subjected to four hours of simulated transport were referred to as Treatments 1, 2, and 3 respectively. Same loading densities subjected to six- hour duration were respectively named Treatments 4, 5 and 6. Percent survival of the fry were monitored daily within 14 days culture period after simulated transport.

Two- Way Analysis of Variance at 0.05α (SPSS, Norusis 1986) revealed that the factors, namely: A) loading density, and B) duration of transport significantly affected percent survival of native catfish fry (Appendix B).

Subjecting three- week old native catfish fry from four to six hours of simulated transport resulted to decline of survival rates within the two-week culture period (Table 1). A day after simulated transport, percent survival of the native catfish fry in all treatment groups decreased to 53-89% (Figures 1-3). At four hours transport duration, decreased in percent survival from day one to day two was from 76.70- 89.36% to 75.20-83.25%. At six hours transport duration, decrease in percent survival from day one to day two was from

Table 1. Percent survival of three- week old native catfish fry, (*Clarias macrocephalus* Gunther) within two weeks after exposure to simulated transport conditions at different loading densities and transport duration. Values are means ± S.E. Different letters attached to values (superscript) denote significant differences (Duncan's test, $p < 0.05$, n=3).

Duration of transport (hours)	Loading density (fry/10L)	% Survival (mean ± S. E.)						
		day 1	day 2	day 3	day 4	day 5	day 6	day 7
4	1000	89.09 ± 0^{a}	83.25 ± 5.84^{a}	57.79 ± 7.67^{a}	57.79 ± 7.67^{a}	55.78 ± 8.07^{a}	53.86 ± 8.86^{a}	51.93 ± 9.96^{a}
	2000	89.36 ± 0^{a}	75.24 ± 1.84^{a}	63.86 ± 3.87^{a}	60.31 ± 3.89^{a}	57.98 ± 2.86^{a}	57.98 ± 2.86^{a}	56.84 ± 1.81^{a}
	3000	76.70 ± 8.31^{ab}	75.20 ± 8.14^{a}	57.81 ± 4.39^{a}	51.15 ± 6.25^{ab}	48.58 ± 7.51^{ab}	45.99 ± 8.72^{ab}	$45.99 \pm 8.72a^{b}$
6	1000	74.70 ± 7.57^{ab}	43.08 ± 1.92^{b}	36.93 ± 5.44^{b}	36.93 ± 5.44^{bc}	28.28 ± 4.94^{bc}	28.28 ± 4.93^{bc}	$26.07 \pm 4.27b^{c}$
	2000	64.17 ± 5.37^{bc}	40.00 ± 5.27^{b}	29.69 ± 3.89^{b}	27.21 ± 4.49^{c}	24.23 ± 5.97^{c}	23.16 ± 5.22^{bc}	$23.16 \pm 5.22b^{c}$
	3000	53.94 ± 8.36^{c}	23.85 ± 11.57^{b}	20.53 ± 10.31^{b}	16.08 ± 10.98^{c}	12.76 ± 12.25^{c}	12.76 ± 12.25^{c}	12.09 ± 11.58^{c}

Con't. Table 2.

Duration of transport (hours)	Loading density (fry/ 10 L)	% Survival (mean ± S. E.)						
		day 8	**day 9**	**day 10**	**day 11**	**day 12**	**day 13**	**day 14**
4	1000	51.98 ± 9.96^{a}	49.22 ± 9.96^{a}	49.22 ± 7.30^{a}	49.22 ± 7.30^{ab}	49.22 ± 7.30^{a}	49.22 ± 7.30^{a}	49.22 ± 7.30^{a}
	2000	54.83 ± 2.72a	54.83 ± 2.72^{a}	54.83 ± 2.72^{a}	54.83 ± 2.72^{a}	54.83 ± 2.72^{a}	53.85 ± 3.08^{a}	53.85 ± 3.08^{a}
	3000	45.99 ± 8.72^{ab}	45.22 ± 7.75^{ab}	44.54 ± 8.31^{ab}	47.54 ± 7.88^{ab}	41.74 ± 6.50^{ab}	41.74 ± 6.50^{ab}	41.06 ± 6.86^{ab}
6	1000	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}	26.07 ± 4.27^{bc}
	2000	23.16 ± 5.22^{bc}	21.90 ± 4.75^{c}	21.90 ± 4.95^{bc}	21.90 ± 4.45^{bc}	21.90 ± 4.954^{bc}	21.90 ± 4.95^{bc}	21.90 ± 4.95^{bc}
	3000	12.09 ± 11.58^{c}	12.09 ± 11.58^{c}	12.09 ± 11.58^{c}	12.09 ± 11.58^{c}	11.40 ± 10.40^{c}	11.41 ± 10.40^{c}	10.70 ± 10.19^{c}

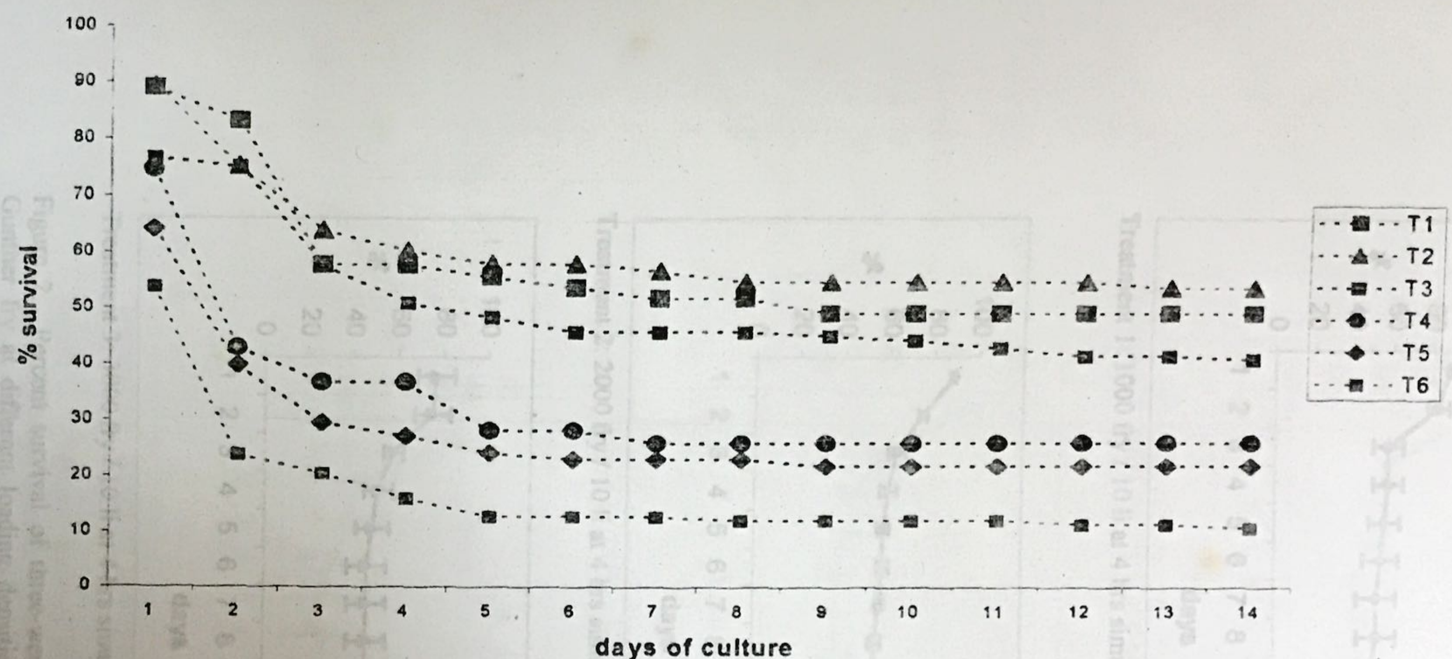
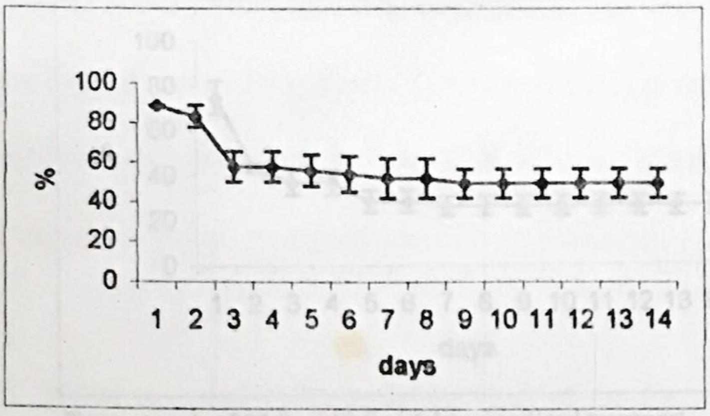
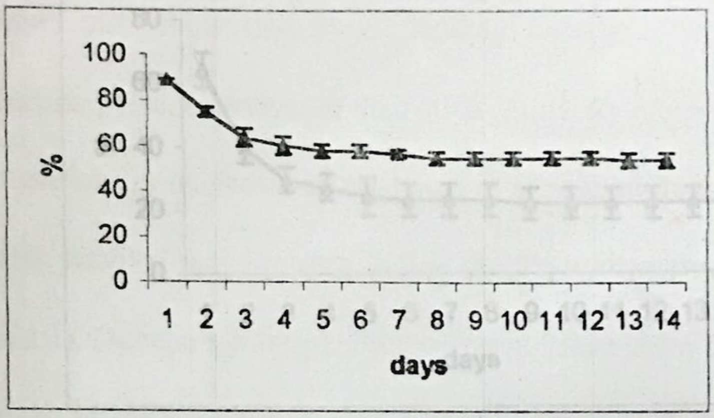


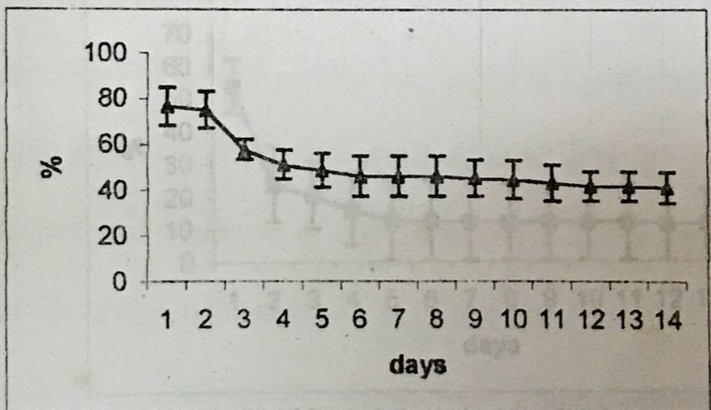
Figure 1. Percent survival of three- week old native catfish fry, *Clarias macrocephalus* Gunther, within two- weeks after exposure to simulated transport conditions at different loading densities and duration of transport. T1, T2, and T3 are loading densities of 1000, 2000, and 3000 fry / 10 li, respectively, subjected to four hours of simulated transport. T4, T5, and T6 are loading densities of 1000, 2000, and 3000 fry / 10 li, respectively, subjected to six hours of simulated transport.



Treatment 1: 1000 fry / 10 li at 4 hrs simulated transport

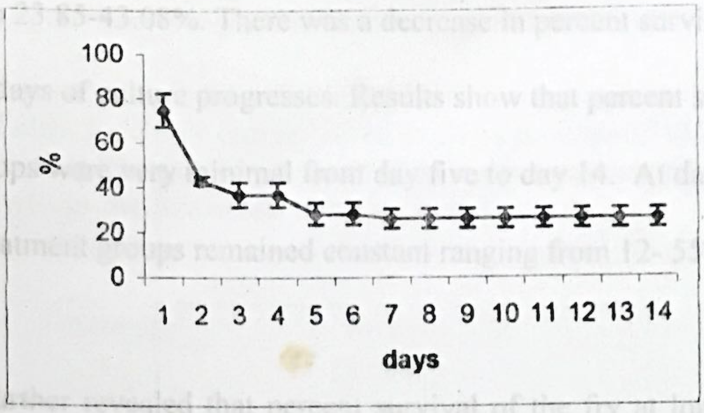


Treatment 2: 2000 fry / 10 li at 4 hrs simulated transport

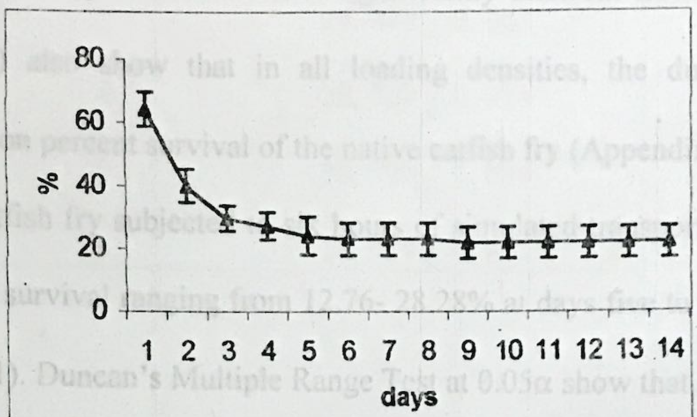


Treatment 3: 3000 fry / 10 li at 4 hrs simulated transport

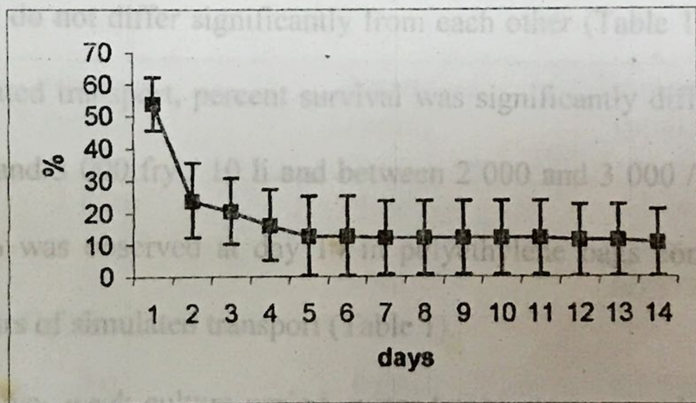
Figure 2. Percent survival of three-week old native catfish, *Clarias macrocephalus* Gunther fry at different loading densities subjected to 4 hrs of simulated transport. Values are means. Bars are standard error of the mean.



Treatment 4: 1000 fry / 10 li at 6 hrs simulated transport



Treatment5: 2000 fry / 10 li at 6 hrs simulated transport



Treatment 6: 3000 fry / 10 li at 6 hrs simulated transport

Figure 3. Percent survival of three-week old native catfish fry, *Clarias macrocephalus* Gunther fry at different loading densities subjected to 6 hrs of simulated transport. Values are means. Bars are standard error of the mean.

53.94- 74.20% to 23.85-43.08%. There was a decrease in percent survival of the fry in all treatment as the days of culture progresses. Results show that percent survival of the fry in all treatment groups were very minimal from day five to day 14. At day nine, percent survival in all treatment groups remained constant ranging from 12- 55% up to day 14 (Figure 1).

Results further revealed that percent survival of the fry at loading densities of 1 000 fry / 10 li and 2 000 fry / 10 li were not significantly different from each other. Analysis of Variance (0.05α) also show that in all loading densities, the duration of transport has significant effect on percent survival of the native catfish fry (Appendix B).

Native catfish fry subjected to six hours of simulated transport in all loading densities have low percent survival ranging from 12.76- 28.28% at days five to nine and 10.70- 26.07% at day 14 (Table 1). Duncan's Multiple Range Test at 0.05α show that daily percent survival of native catfish fry at loading densities of 1 000, 2 000 and 3 000 / 10 li at four hours of simulated transport do not differ significantly from each other (Table 1). For fry subjected to six hours of simulated transport, percent survival was significantly different between loading densities of 1 000 and 3 000 fry / 10 li and between 2 000 and 3 000 / 10 li. Lowest percent survival of 10.70% was observed at day 14 in polyethylene bags containing 3 000 / 10 li subjected to six hours of simulated transport (Table 1).

Within the two- week culture period, water temperature ranged from 28. 71 – 28.9°C (Table 2). Ranges in nitrite and ammonia were 0-0.005 ppm and 0-4.370 ppm, respectively (Table 3).

Table 2. Water temperature in all experimental set- ups. Values are means ± S.D. of daily measurements taken within the two-week culture period (n = 3).

Treatment	Mean ± S.D.
1	29. 048 ± 0.113
2	29. 089 ± 0. 093
3	29. 048 ± 0. 063
4	28. 708 ± 0. 021
5	28. 798 ± 0. 074
6	28. 929 ± 0. 071

Table 3. Nitrite and ammonia content of water (ppm) one hour and 24 hours after the four- hour simulated transport condition.
Nd - not detectable

Stocking density	Nitrite (ppm)	Nitrite (ppm)	Ammonia (ppm)	Ammonia (ppm)
(ind. / 10 li)	1 hour	24 hours	1 hour	24 hours
1 000 ind. / 10 li	Nd	Nd	0.7577	1.0496
2 000 ind. / 10 li	Nd	0.001	0.9957	1.2377
3 000 ind. / 10 li	Nd	0.004	0.7778	3.1217

To summarize the present results indicate that loading density and duration of transport influence the viability of three- week old native catfish, *C. macrocephalus* Gunther, fry subjected to simulated transport. The optimum loading densities resulting in high survival rates can be 1 000 up to 2 000 ind./ 10 li. However, the duration of transport must not exceed four hours. Loading density during transport of native catfish must be less than 1 000 ind./ 10 li if the duration of transport will be more than four hours.

B. Discussion

The present results indicate that the loading density and duration of transport significantly affect the viability of three- week old native catfish *Clarias macrocephalus* Gunther fry. The optimum loading density that resulted to high survival rate is 1 000 and 2 000 fry / 10 li.

The low survival rate noted at density of 3 000 fry / 10 li may have been caused by accumulation of toxins in the transport water, oxygen starvation, hyperactivity, straining, exhaustion and other accidents due to overcrowding and starvation (Ramachandran, 1969).

Furthermore, prolonging the duration of simulated transport affected the percent survival of the fish (Table 1). Six hours of transport resulted to significantly lower survival rates (10 – 26%) in all loading densities compared to those subjected to four hours of transport (41- 54%).

Metabolic waste content of water, specifically ammonia and nitrite, was determined an hour and a day after simulated transport conditions. Nitrite content is usually non-detectable or present in very low concentration while ammonia content is higher. Based on resulting data, the fish can tolerate up to 2.406 ppm of ammonia. Nitrite content in loading densities 1 000, 2 000 and 3 000 ind. / 10 li was non-detectable. Ammonia content in these loading densities is 0.7577- 0.9957 ppm. After one day, ammonia content rised to 1.0496- 3.1217 ppm.

CHAPTER 5

SUMMARY OF SIGNIFICANT FINDINGS, CONCLUSION AND RECOMMENDATION

A. Summary of Significant Findings

The researchers have found that the optimum loading density for three- week old native catfish *C. macrocephalus* Gunther subjected to simulated transport conditions is 1 000- 2 000 individuals / 10 li, with the duration of transport of only four hours or less. Loading densities of 1 000, 2000 and 3 000 fry / 10 li, respectively subjected to four hours of simulated transport were labeled as treatments 1, 2 and 3. Treatments 4, 5 and 6 are loading densities of 1 000, 2 000 and 3 000 / 10 li, respectively, subjected to six hours of simulated transport. Survival rates in treatments 1,2 and 3 were not significantly different from each other (55- 65 %) ($P > 0.05$). Survival rates observed in treatments 4, 5 and 6, which were subjected to six- hour transport duration, were significantly lower (10- 28%) ($P < 0.05$).

B. Conclusion

The researchers concluded that loading density and duration of transport do affect the survival rate of three- week old native catfish (*C. macrocephalus* Gunther) fry subjected to simulated transport conditions. Increasing the loading density and duration of transport result to lower percent survival of the native catfish fry within the two- week culture period.

Based on the results of this study, it can be concluded that the fry should be transported with loading density below 3 000 fry / 10 li and should not exceed four hours of transport duration. It is not known entirely under what condition native catfish can have highest survival rate. Further research should be done to determine this.

C. Recommendations

The researchers recommend loading densities of 1 000 to 2 000 fry / 10 li to those who want to transport native catfish fry. This may also apply to other species of fish. However, the duration of transport must not exceed four hours. If the transport duration goes beyond four hours, we recommend loading density that is lesser than 1 000 fry / 10 li.

If sometime later, other students will undertake this kind of study, the researchers highly recommend the following:

1. Use various stages of the fish in order to determine what is the best stage for the fish to be transported in order to have higher percent survival.

2. Try to simulate transport in different temperatures to find out if it does affect the percent survival of the fish.

3. Subject a set of fish that have eaten and another set that have not eaten to simulated transport in order to determine if food (metabolic waste) has an effect on its survival rate.

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Plates:

Plate 1. **Collecting three-week old native catfish fry at larval rearing tank**

Plate 2. **Inflation of plastic bags to be subjected to simulated transport**

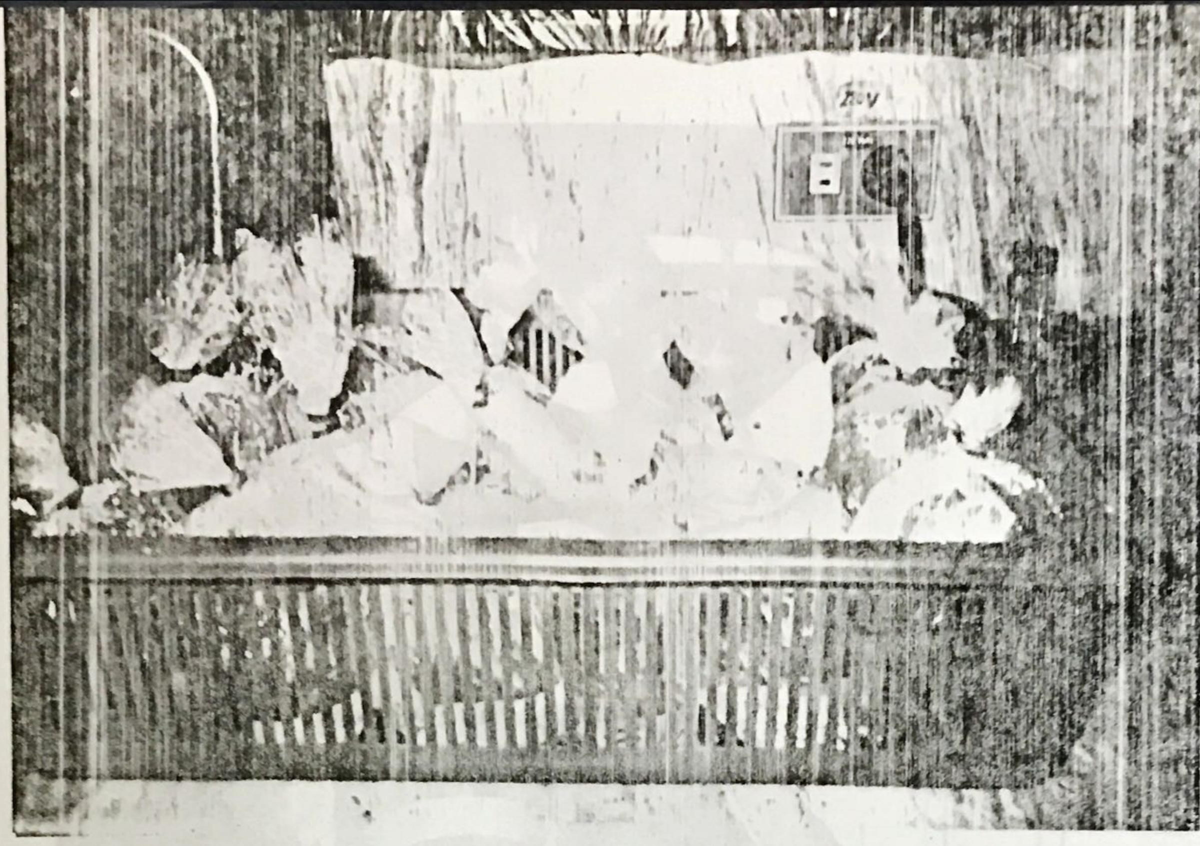

Plate 3. Sealed polyethylene bags ready for simulated transport

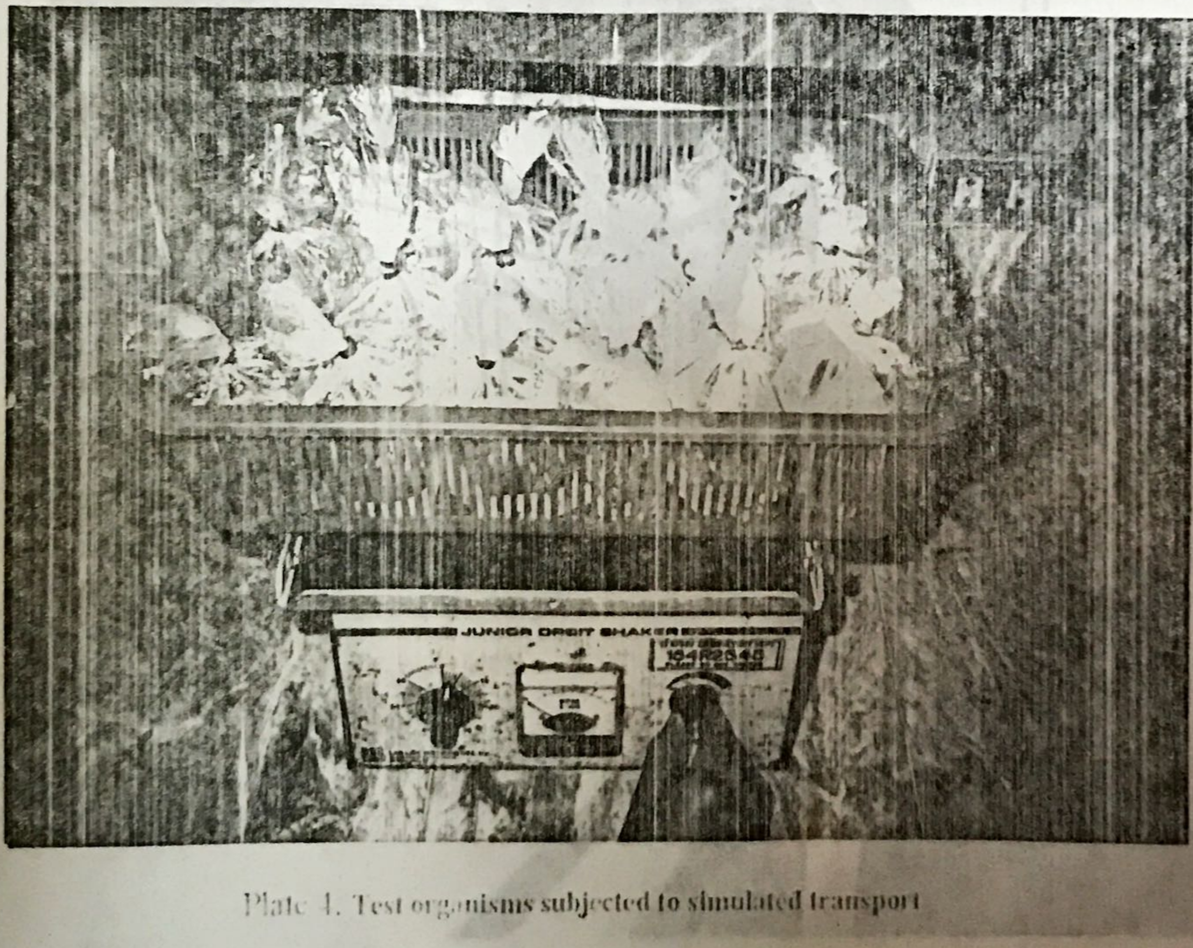

Plate 4. Test organisms subjected to simulated transport

Plate 5. Daily changing of water in each set-up

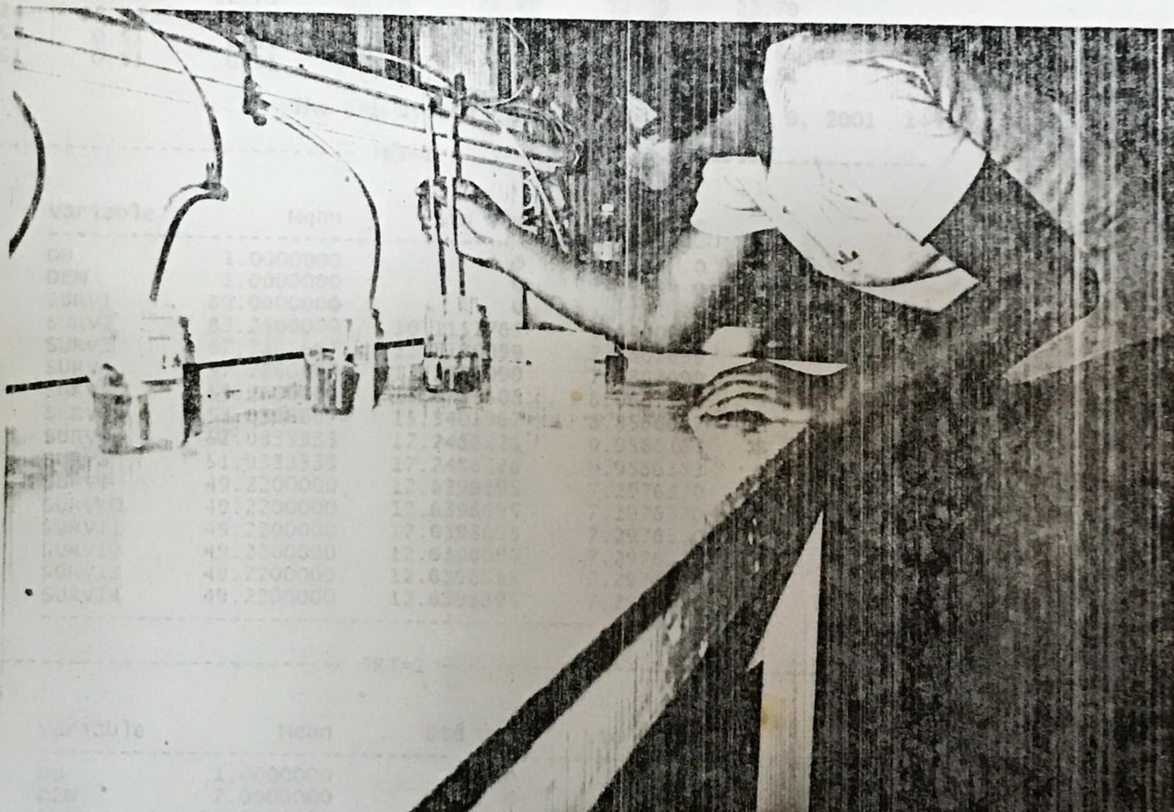

Plate 6. The researcher measuring the temperature

SURV14	53.8466667	5.3289430	3.0766667

TRT=3

Variable	Mean	Std Dev	Std Error
DU	1.0000000	0	0
DEN	3.0000000	0	0
SURV1	76.6966667	14.3848682	8.3051075
SURV2	75.2000000	14.1810578	8.1874375
SURV3	57.8133333	7.6068018	4.3917891
SURV4	51.1466667	10.8316035	6.2536292
SURV5	48.5833333	13.0016358	7.5064979
SURV6	45.9900000	15.1034830	8.7200000
SURV7	45.9900000	15.1034830	8.7200000
SURV8	45.9900000	15.1034830	8.7200000
SURV9	45.2200000	13.7698039	7.9500000
SURV10	44.5433333	14.3916515	8.3090239
SURV11	43.1233333	13.6543339	7.8833333
SURV12	41.7400000	11.2583302	6.5000000
SURV13	41.7400000	11.2583302	6.5000000
SURV14	41.0633333	11.8877514	6.8633965

TRT=4

Variable	Mean	Std Dev	Std Error
DU	2.0000000	0	0
DEN	1.0000000	0	0
SURV1	74.6966667	13.1126250	7.5705776
SURV2	43.0766667	3.3313111	1.9233333
SURV3	36.9333333	9.4272071	5.4428005
SURV4	36.9333333	9.4272071	5.4428005
SURV5	28.2833333	8.5332370	4.9266667
SURV6	28.2833333	8.5332370	4.9266667
SURV7	26.0700000	7.4026752	4.2739365
SURV8	26.0700000	7.4026752	4.2739365
SURV9	26.0700000	7.4026752	4.2739365
SURV10	26.0700000	7.4026752	4.2739365
SURV11	26.0700000	7.4026752	4.2739365
SURV12	26.0700000	7.4026752	4.2739365
SURV13	26.0700000	7.4026752	4.2739365
SURV14	26.0700000	7.4026752	4.2739365

TRT=5

Variable	Mean	Std Dev	Std Error
DU	2.0000000	0	0
DEN	2.0000000	0	0
SURV1	64.1700000	9.3004086	5.3695934
SURV2	40.0000000	9.1234259	5.2674124
SURV3	29.6866667	6.7454602	3.8944933
SURV4	27.2133333	7.7740744	4.4883640
SURV5	24.2333333	10.3448554	5.9726051
SURV6	23.1633333	9.0352440	5.2165005
SURV7	23.1633333	9.0352440	5.2165005
SURV8	23.1633333	9.0352440	5.2165005
SURV9	21.9033333	8.5744524	4.9504624
SURV10	21.9033333	8.5744524	4.9504624
SURV11	21.9033333	8.5744524	4.9504624
SURV12	21.9033333	8.5744524	4.9504624
SURV13	21.9033333	8.5744524	4.9504624
SURV14	21.9033333	8.5744524	4.9504624

TRT=6

Variable	Mean	Std Dev	Std Error
DU	2.0000000	0	0
DEN	3.0000000	0	0
SURV1	53.9400000	14.4827725	8.3616326
SURV2	23.8466667	20.0421414	11.5713358
SURV3	20.5300000	17.8591937	10.3110103
SURV4	16.0833333	19.0120102	10.9765892
SURV5	12.7633333	21.2233959	12.2533333
SURV6	12.7633333	21.2233959	12.2533333
SURV7	12.0866667	20.0513748	11.5766667

SURV8	12.0866667	20.0513748	11.5766667
SURV9	12.0866667	20.0513748	11.5766667
SURV10	12.0866667	20.0513748	11.5766667
SURV11	12.0866667	20.0513748	11.5766667
SURV12	11.4100000	18.8793538	10.9000000
SURV13	11.4100000	18.8793538	10.9000000
SURV14	10.7033333	17.6553712	10.1933333

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Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TRT	6	1 2 3 4 5 6

Number of observations in data set = 18

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Analysis of Variance Procedure

Dependent Variable: SURV1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2903.46824444	580.69364889	5.16	0.0093
Error	12	1350.22733333	112.51894444		
Corrected Total	17	4253.69557778			

R-Square	C.V.	Root MSE	SURV1 Mean
0.682575	14.20795	10.60749473	74.65888889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	2903.46824444	580.69364889	5.16	0.0093

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 112.5189

Number of Means	2	3	4	5	6
Critical Range	18.87	19.75	20.29	20.64	20.88

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRT
	A	89.360	3	2
	A	89.090	3	1
B	A	76.697	3	3
B	A	74.697	3	4
B	C	64.170	3	5
	C	53.940	3	6

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Analysis of Variance Procedure
Class Level Information

Class Levels Values

Duncan Grouping	Mean	N	TRT
A			
A	32.49	3	6
A			
A	31.18	3	2
A			
A	26.76	3	3

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Analysis of Variance Procedure

Dependent Variable: SURV13

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1755.14556111	351.02911222	0.67	0.6509
Error	12	6246.90040000	520.57503333		
Corrected Total	17	8002.04596111			

R-Square	C.V.	Root MSE	SURV13 Mean
0.219337	61.82763	22.81611346	36.90277778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1755.14556111	351.02911222	0.67	0.6509

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV13

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 520.575

Number of Means	2	3	4	5	6
Critical Range	40.59	42.49	43.63	44.39	44.92

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A			
A	46.84	3	5
A			
A	32.49	3	6
A			
A	31.18	3	2
A			
A	30.29	3	1
A			
A	26.76	3	3

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Analysis of Variance Procedure

Dependent Variable: SURV14

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1582.72237778	316.54447556	0.63	0.6840
Error	12	6076.15033333	506.34586111		
Corrected Total	17	7658.87271111			

R-Square	C.V.	Root MSE	SURV14 Mean
0.206652	61.76436	22.50213015	36.43222222

TRT	5	1624.92477778	324.98495556	0.63	0.6809

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV11

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 516.0036

Number of Means	2	3	4	5	6
Critical Range	40.41	42.30	43.44	44.20	44.72

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A			
A	46.84	3	5
A			
A	34.93	3	1
A			
A	32.49	3	6
A			
A	31.18	3	2
A			
A	26.76	3	3

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Analysis of Variance Procedure

Dependent Variable: SURV12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1665.89498333	333.17899667	0.66	0.6634
Error	12	6097.99266667	508.16605556		
Corrected Total	17	7763.88765000			

R-Square	C.V.	Root MSE	SURV12 Mean
0.214570	60.34678	22.54253880	37.35500000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1665.89498333	333.17899667	0.66	0.6634

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV12

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 508.1661

Number of Means	2	3	4	5	6
Critical Range	40.10	41.98	43.11	43.86	44.38

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A			
A	46.84	3	5
A			
A	33.00	3	1

Duncan Grouping	Mean	N	TRT
A			
A	34.78	3	6
A			
A	33.12	3	2

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Analysis of Variance Procedure

Dependent Variable: SURV10

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1535.00844444	307.00168889	0.55	0.7354
Error	12	6690.45406667	557.53783889		
Corrected Total	17	8225.46251111			

R-Square	C.V.	Root MSE	SURV10 Mean
0.186617	60.26947	23.61223918	39.17777778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1535.00844444	307.00168889	0.55	0.7354

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV10

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 557.5378

Number of Means	2	3	4	5	6
Critical Range	42.01	43.97	45.16	45.94	46.49

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A			
A	47.91	3	5
A			
A	40.86	3	1
A			
A	32.49	3	6
A			
A	31.18	3	2
A			
A	28.76	3	3

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Analysis of Variance Procedure

Dependent Variable: SURV11

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1624.92477778	324.98495556	0.63	0.6809
Error	12	6192.04366667	516.00363889		
Corrected Total	17	7816.96844444			

R-Square	C.V.	Root MSE	SURV11 Mean
0.207871	60.29298	22.71571348	37.67555556

Source	DF	Anova SS	Mean Square	F Value	Pr > F

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV8

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 507.8834

Number of Means	2	3	4	5	6
Critical Range	40.09	41.96	43.10	43.85	44.37

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A	48.93	3	5
A	42.79	3	1
A	39.66	3	3
A	34.78	3	6
A	34.14	3	2

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Analysis of Variance Procedure

Dependent Variable: SURV9

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1005.01782778	201.00356556	0.40	0.8407
Error	12	6052.89653333	504.40804444		
Corrected Total	17	7057.91436111			

R-Square	C.V.	Root MSE	SURV9 Mean
0.142396	53.55393	22.45903035	41.93722222

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1005.01782778	201.00356556	0.40	0.8407

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV9

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 504.408

Number of Means	2	3	4	5	6
Critical Range	39.95	41.82	42.95	43.70	44.22

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	53.86	3	4
A	48.93	3	5
A	42.79	3	1
A	38.15	3	3

A	36.45	3	6
A			
A	36.36	3	2

Analysis of Variance Procedure

Dependent Variable: SURV7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1192.32965000	238.46593000	0.44	0.8129
Error	12	6514.70320000	542.89193333		
Corrected Total	17	7707.03285000			

R-Square	C.V.	Root MSE	SURV7 Mean
0.154707	52.98073	23.30004149	43.97833333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1192.32965000	238.46593000	0.44	0.8129

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV7

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 542.8919

Number of Means	2	3	4	5	6
Critical Range	41.45	43.39	44.56	45.34	45.88

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	58.08	3	4
A			
A	50.00	3	5
A			
A	45.00	3	1
A			
A	39.66	3	3
A			
A	36.36	3	2
A			
A	34.78	3	6

Analysis of Variance Procedure

Dependent Variable: SURV8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	923.39491111	184.67898222	0.36	0.8638
Error	12	6094.60086667	507.88340556		
Corrected Total	17	7017.99577778			

R-Square	C.V.	Root MSE	SURV8 Mean
0.131575	53.20316	22.53626867	42.35888889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	923.39491111	184.67898222	0.36	0.8638

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV5

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 703.0014

Number of Means	2	3	4	5	6
Critical Range	47.17	49.37	50.71	51.59	52.20

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	66.63	3	4
A	55.69	3	5
A	49.93	3	1
A	39.66	3	3
A	38.28	3	2
A	37.59	3	6

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Analysis of Variance Procedure

Dependent Variable: SURV6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1226.82211667	245.36442333	0.43	0.8212
Error	12	6891.97933333	574.33161111		
Corrected Total	17	8118.80145000			

R-Square	C.V.	Root MSE	SURV6 Mean
0.151109	52.94036	23.96521669	45.26833333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	1226.82211667	245.36442333	0.43	0.8212

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV6

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 574.3316

Number of Means	2	3	4	5	6
Critical Range	42.63	44.63	45.83	46.63	47.18

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	58.08	3	4
A	51.14	3	5
A	49.93	3	1
A	39.66	3	3

```
                          A
                          A                    38.25      3   6

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                     Analysis of Variance Procedure

Dependent Variable: SURV4

Source              DF      Sum of Squares        Mean Square   F Value      Pr > F
Model                5      2515.85684444        503.17136889      0.72      0.6235
Error               12      8432.96833333        702.74736111
Corrected Total     17     10948.82517778

            R-Square              C.V.          Root MSE             SURV4 Mean
            0.229783          53.29709       26.50938251            49.73888889

Source              DF          Anova SS        Mean Square   F Value      Pr > F
TRT                  5      2515.85684444        503.17136889      0.72      0.6235

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                     Analysis of Variance Procedure

            Duncan's Multiple Range Test for variable: SURV4

        NOTE: This test controls the type I comparisonwise error rate, not the
              experimentwise error rate

                   Alpha= 0.05  df= 12  MSE= 702.7474

            Number of Means      2     3     4     5     6
            Critical Range   47.16 49.36 50.70 51.58 52.19

        Means with the same letter are not significantly different.

            Duncan Grouping                Mean      N  TRT

                          A               68.55      3   4
                          A
                          A               59.79      3   5
                          A
                          A               53.86      3   1
                          A
                          A               40.36      3   3
                          A
                          A               38.28      3   2
                          A
                          A               37.59      3   6

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                     Analysis of Variance Procedure

Dependent Variable: SURV5

Source              DF      Sum of Squares        Mean Square   F Value      Pr > F
Model                5      2047.06973333        409.41394667      0.58      0.7134
Error               12      8436.01626667        703.00135556
Corrected Total     17     10483.08600000

            R-Square              C.V.          Root MSE             SURV5 Mean
            0.195274          55.28009       26.51417273            47.96333333

Source              DF          Anova SS        Mean Square   F Value      Pr > F
TRT                  5      2047.06973333        409.41394667      0.58      0.7134
```

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV2

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 495.6129

Number of Means	2	3	4	5	6
Critical Range	39.60	41.45	42.58	43.32	43.83

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean    N  TRT

            A         89.09    3  4
            A
      B     A         83.25    3  1
      B     A
      B     A         68.15    3  5
      B     A
      B     A         56.68    3  2
      B     A
      B     A         53.71    3  3
      B
      B               43.07    3  6
```

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Analysis of Variance Procedure

Dependent Variable: SURV3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2514.41969444	502.88393889	0.82	0.5584
Error	12	7359.54820000	613.29568333		
Corrected Total	17	9873.96789444			

R-Square	C.V.	Root MSE	SURV3 Mean
0.254651	46.58590	24.76480736	53.15944444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	2514.41969444	502.88393889	0.82	0.5584

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV3

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 613.2957

Number of Means	2	3	4	5	6
Critical Range	44.06	46.11	47.36	48.19	48.76

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean    N  TRT

            A         70.48    3  4
            A
            A         61.92    3  1
            A
            A         60.81    3  5
            A
            A
            A         45.09    3  2
            A
            A         42.42    3  3
```

```
B          A           41.063      3  3
B
B          C           26.070      3  4
B          C
B          C           21.903      3  5
           C
           C           10.703      3  6
```

```
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          Analysis of Variance Procedure

    Duncan's Multiple Range Test for variable: SURV1

NOTE: This test controls the type I comparisonwise error rate, not the
      experimentwise error rate

        Alpha= 0.05  df= 12  MSE= 398.9485

     Number of Means      2     3     4     5     6
     Critical Range   35.53 37.19 38.20 38.86 39.33

Means with the same letter are not significantly different.

     Duncan Grouping              Mean       N  TRT

                   A             89.09       3  4
                   A
            B      A             83.25       3  1
            B      A
            B      A             79.34       3  2
            B      A
            B      A             73.64       3  5
            B      A
            B      A             69.67       3  3
            B
            B                    45.00       3  6
```

```
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          Analysis of Variance Procedure
             Class Level Information

          Class     Levels    Values

          TRT            6    1 2 3 4 5 6

     Number of observations in data set = 18
```

```
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          Analysis of Variance Procedure

Dependent Variable: SURV2
```

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4794.23177778	958.84635556	1.93	0.1619
Error	12	5947.35440000	495.61286667		
Corrected Total	17	10741.58617778			

R-Square	C.V.	Root MSE	SURV2 Mean
0.446324	33.90609	22.26236435	65.65888889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4794.23177778	958.84635556	1.93	0.1619

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4214.88751667	842.97750333	6.32	0.0042

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV13

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 133.2775

Number of Means	2	3	4	5	6
Critical Range	20.54	21.50	22.08	22.46	22.73

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N  TRT

              A         53.847     3  2
              A
              A         49.220     3  1
              A
         B    A         41.740     3  3
         B
         B    C         26.070     3  4
         B    C
         B    C         21.903     3  5
              C
              C         11.410     3  6
```


Analysis of Variance Procedure

Dependent Variable: SURV14

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4281.42251111	856.28450222	6.68	0.0034
Error	12	1539.03106667	128.25258889		
Corrected Total	17	5820.45357778			

R-Square	C.V.	Root MSE	SURV14 Mean
0.735582	33.50442	11.32486595	33.80111111

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4281.42251111	856.28450222	6.68	0.0034

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV14

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 128.2526

Number of Means	2	3	4	5	6
Critical Range	20.15	21.09	21.66	22.04	22.30

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N  TRT

              A         53.847     3  2
              A
              A         49.220     3  1
              A
```

```
         B
         B         C                 26.070      3  4
         B         C
         B         C                 21.903      3  5
                   C
                   C                 12.087      3  6
```

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Analysis of Variance Procedure

Dependent Variable: SURV12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4334.62609444	866.92521889	6.56	0.0037
Error	12	1586.95673333	132.24639444		
Corrected Total	17	5921.58282778			

R-Square	C.V.	Root MSE	SURV12 Mean
0.732005	33.62910	11.49984324	34.19611111

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4334.62609444	866.92521889	6.56	0.0037

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV12

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 132.2464

Number of Means	2	3	4	5	6
Critical Range	20.46	21.41	21.99	22.38	22.[illegible]4

Means with the same letter are not significantly different.

```
    Duncan Grouping            Mean      N  TRT

                   A         54.833      3  2
                   A
                   A         49.220      3  1
                   A
         B         A         41.740      3  3
         B
         B         C         26.070      3  4
         B         C
         B         C         21.903      3  5
                   C
                   C         11.410      3  6
```

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Analysis of Variance Procedure

Dependent Variable: SURV13

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4214.88751667	842.97750333	6.32	0.0042
Error	12	1599.32953333	133.27746111		
Corrected Total	17	5814.21705000			

R-Square	C.V.	Root MSE	SURV13 Mean
0.724928	33.92307	11.54458579	34.03166667

TRT	5	4387.89716111	877.57943222	5.73	0.0063

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV10

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 153.2459

Number of Means	2	3	4	5	6
Critical Range	22.02	23.05	23.67	24.09	24.37

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRT
	A	54.83	3	2
	A			
	A	49.22	3	1
	A			
B	A	44.54	3	3
B				
B	C	26.07	3	4
B	C			
B	C	21.90	3	5
	C			
	C	12.09	3	6

Analysis of Variance Procedure

Dependent Variable: SURV11

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4309.72142778	861.94428556	5.75	0.0062
Error	12	1797.59366667	149.79947222		
Corrected Total	17	6107.31509444			

R-Square	C.V.	Root MSE	SURV11 Mean
0.705665	35.43560	12.23925946	34.53944444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4309.72142778	861.94428556	5.75	0.0062

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV11

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 149.7995

Number of Means	2	3	4	5	6
Critical Range	21.77	22.79	23.41	23.81	24.10

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRT
	A	54.833	3	2
	A			
	A	49.220	3	1
	A			
B	A	43.123	3	3

```
                       B         C
                       B         C                  23.16       3  5
                                 C
                                 C                  12.09       3  6
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                        Analysis of Variance Procedure

Dependent Variable: SURV9

Source                 DF       Sum of Squares      Mean Square   F Value      Pr > F
Model                   5         4428.69677778     885.73935556      5.89      0.0056
Error                  12         1803.92700000     150.32725000
Corrected Total        17         6232.62377778

                 R-Square                  C.V.         Root MSE             SURV9 Mean
                 0.710567              35.14242      12.26080136            34.88888889

Source                 DF            Anova SS       Mean Square   F Value      Pr > F
TRT                     5         4428.69677778     885.73935556      5.89      0.0056

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                        Analysis of Variance Procedure
                 Duncan's Multiple Range Test for variable: SURV9
              NOTE: This test controls the type I comparisonwise error rate, not the
                    experimentwise error rate

                      Alpha= 0.05  df= 12  MSE= 150.3272

                   Number of Means      2     3     4     5     6
                   Critical Range   21.81 22.83 23.45 23.86 24.14
              Means with the same letter are not significantly different.
                   Duncan Grouping              Mean      N  TRT
                                 A              54.83     3  2
                                 A
                                 A              49.22     3  1
                                 A
                       B         A              45.22     3  3
                       B
                       B         C              26.07     3  4
                                 C
                                 C              21.90     3  5
                                 C
                                 C              12.09     3  6

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                        Analysis of Variance Procedure

Dependent Variable: SURV10

Source                 DF       Sum of Squares      Mean Square   F Value      Pr > F
Model                   5         4387.89716111     877.57943222      5.73      0.0063
Error                  12         1838.95126667     153.24593889
Corrected Total        17         6226.84842778

                 R-Square                  C.V.         Root MSE            SURV10 Mean
                 0.704674              35.59701      12.37925433            34.77611111

Source                 DF            Anova SS       Mean Square   F Value      Pr > F
```

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV7

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 178.9934

Number of Means	2	3	4	5	6
Critical Range	23.80	24.91	25.59	26.03	26.34

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N  TRT

              A         56.84      3  2
              A
              A         51.93      3  1
              A
         B    A         45.99      3  3
         B
         B    C         26.07      3  4
         B    C
         B    C         23.16      3  5
              C
              C         12.09      3  6
```

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Analysis of Variance Procedure

Dependent Variable: SURV8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4628.94462778	925.78892556	5.11	0.0097
Error	12	2172.68306667	181.05692222		
Corrected Total	17	6801.62769444			

R-Square	C.V.	Root MSE	SURV8 Mean
0.680564	37.71286	13.45573938	35.67944444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4628.94462778	925.78892556	5.11	0.0097

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Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV8

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 181.0569

Number of Means	2	3	4	5	6
Critical Range	23.94	25.06	25.73	26.18	26.49

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N  TRT

              A         54.83      3  2
              A
              A         51.93      3  1
              A
         B    A         45.99      3  3
         B
         B    C         26.07      3  4
```

Analysis of Variance Procedure

Dependent Variable: SURV7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4869.62422778	973.92484556	5.44	0.0076
Error	12	2147.92080000	178.99340000		
Corrected Total	17	7017.54502778			

R-Square	C.V.	Root MSE	SURV7 Mean
0.693921	37.14912	13.37884150	36.01388889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4869.62422778	973.92484556	5.44	0.0076

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV6

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 182.1519

Number of Means	2	3	4	5	6
Critical Range	24.01	25.13	25.81	26.26	26.57

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRT
	A	57.98	3	2
	A	53.86	3	1
B	A	45.99	3	3
B	C	28.28	3	4
	C	23.16	3	5
	C	12.76	3	6

Analysis of Variance Procedure

Dependent Variable: SURV6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4980.35086667	996.07017333	5.47	0.0075
Error	12	2185.82333333	182.15194444		
Corrected Total	17	7166.17420000			

R-Square	C.V.	Root MSE	SURV6 Mean
0.694980	36.47010	13.49636782	37.00666667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4980.35086667	996.07017333	5.47	0.0075

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV5

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 169.869

Number of Means	2	3	4	5	6
Critical Range	23.19	24.27	24.93	25.36	25.66

Means with the same letter are not significantly different.

```
Duncan Grouping          Mean      N  TRT

                 A       57.98     3  2
                 A
                 A       55.78     3  1
                 A
            B    A       48.58     3  3
            B
            B    C       28.28     3  4
                 C
                 C       24.23     3  5
                 C
                 C       12.76     3  6
```

Analysis of Variance Procedure

Dependent Variable: SURV5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5244.80937778	1048.96187556	6.18	0.0047
Error	12	2038.42813333	169.86901111		
Corrected Total	17	7283.23751111			

R-Square	C.V.	Root MSE	SURV5 Mean
0.720121	34.35462	13.03338065	37.93777778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	5244.80937778	1048.96187556	6.18	0.0047

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV4

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 141.8044

Number of Means	2	3	4	5	6
Critical Range	21.18	22.17	22.77	23.17	23.45

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TRT
	A	60.313	3	2
	A			
B	A	57.787	3	1
B	A			
B	A	51.147	3	3
B				
B	C	36.933	3	4
	C			
	C	27.213	3	5
	C			
	C	16.083	3	6

Analysis of Variance Procedure

Dependent Variable: SURV4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4749.56269444	949.91253889	6.70	0.0034
Error	12	1701.65220000	141.80435000		
Corrected Total	17	6451.21489444			

R-Square	C.V.	Root MSE	SURV4 Mean
0.736228	28.63954	11.90816317	41.57944444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4749.56269444	949.91253889	6.70	0.0034

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV3

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 122.1743

Number of Means	2	3	4	5	6
Critical Range	19.66	20.58	21.14	21.51	21.76

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	63.857	3	2
A			
A	57.813	3	3
A			
A	57.787	3	1
B	36.933	3	4
B			
B	29.687	3	5
B			
B	20.550	3	6

TRT 6 1 2 3 4 5 6

Number of observations in data set = 18

Analysis of Variance Procedure

Dependent Variable: SURV2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	8804.41649444	1760.88329889	13.05	0.0002
Error	12	1619.12240000	134.92686667		
Corrected Total	17	10423.53889444			

R-Square	C.V.	Root MSE	SURV2 Mean
0.844667	20.46136	11.61580245	56.76944444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	8804.41649444	1760.88329889	13.05	0.0002

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: SURV2

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 12 MSE= 134.9269

Number of Means	2	3	4	5	6
Critical Range	20.66	21.63	22.21	22.60	22.87

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	83.250	3	1
A			
A	75.243	3	2
A			
A	75.200	3	3
B	43.077	3	4
B			
B	40.000	3	5
B			
B	23.847	3	6

Analysis of Variance Procedure

Dependent Variable: SURV3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4739.05591111	947.81118222	7.76	0.0018
Error	12	1466.09113333	122.17426111		
Corrected Total	17	6205.14704444			

R-Square	C.V.	Root MSE	SURV3 Mean
0.763730	24.87540	11.05324663	44.43444444

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	4739.05591111	947.81118222	7.76	0.0018