

## THE USE OF *Cocos nucifera* L. WATER AS MILT EXTENDER IN ARTIFICIAL BREEDING OF *Clarias gariepinus* (BURCHELL, 1822)

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### ABSTRACT

The efficacy of coconut water as milt extender in artificial propagation of *Clarias gariepinus* was examined. Coconut water obtained from immature and mature Coconut fruits were used as the tested extenders while, the physiological solution was considered as the control experiment. The milt was added to the extenders at two different dilution ratios (1:10 and 1:20) and replicated thrice. Each extender was added to 700 eggs and allowed to stay for about 5 minutes, after which the eggs were evenly spread on the prepared spawning nets for incubation at water temperature of 26-27°C. The fertilization rate was examined 2 hours after the eggs were immersed in water, the hatching rate was monitored 28 hours after fertilization and the survival rate was determined on the 14<sup>th</sup> day after hatching. The data collected were analysed using one-way ANOVA, while the differences between the means were compared using Duncan multiple range test. The results showed that the extenders had significant effects ( $P < 0.05$ ) on the fertilization, hatching and survival rates of *Clarias gariepinus*. The production cost of coconut water was lower than physiological solution and that mature coconut water at ratio 1:20 served as the best milt extender in the artificial breeding of *Clarias gariepinus*.

**Keywords:** Saline solution, Cost implication, Hypophysation.

### Introduction

The development of aquaculture in Nigeria and other developing countries has been hindered by the inadequate supply of good quality fish seeds among other factors. Successful aquaculture business begins with stocking good quality fingerling fish with optimum conditions which enhance rapid growth and harvest within short possible time (Adewolu *et al.*, 2008). Therefore, to increase the number and improve the quality of fish seeds produced, efforts are being geared towards improving the fish seeds production through the use of extenders with the ultimate goal of reducing cost of production. Extender has been described as the medium which increases the contact areas between eggs and spermatozoa in an induced breeding (Muchlisin *et al.*, 2010). The highly viscous and small volume of milt generally produced by matured male fish necessitate extenders which are required to dilute the milt so as to increase its contact areas, thus increasing the fertilization rate during artificial breeding of fish. Adewolu *et al.* (2008) reported *Clarias gariepinus* as one of the most important freshwater fish species currently cultured within and outside of tropical and subtropical environment. This species is widely accepted by Nigerians because of its positive attributes which include: its hardy nature, good taste, ability to grow fast among others (Olaleye, 2005).

The coconut (*Cocos nucifera* L.) is an important fruit tree in the tropical regions. It contains the edible fruit (coconut meat) and the coconut water (Jean *et al.*, 2009). Coconut water is the clear liquid found inside young green coconut (immature coconut). Coconut water serves as the aqueous suspension for the coconut endosperm during the nuclear phase of development. As the

coconut plant develops, its cellular endosperm becomes translucent and jelly-like, but later hardens at maturity to become white flesh (coconut meat). George and Sherrington (1984) reported that coconut water contains growth regulatory properties which include cytokinin- type activity. Also present in the coconut water are: sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzyme (Jean *et al.*, 2009). Coconut water is rich in low levels of fat, carbohydrates and electrolytes such as potassium, sodium, calcium and magnesium (Saat *et al.*, 2002). The concentrations of these electrolytes in coconut water produce an osmotic pressure which is similar to that observed in blood (Fernandes *et al.*, 2000). However, coconut water has been reportedly used as an intravenous rehydration fluid instead of saline solution during World War II (Weimar, 2011). Coconut water also contains B vitamins which are required as co-enzymes for enzymatic reactions for cellular function (Depeint *et al.*, 2006). Vitamin C which is an important dietary antioxidant is also present in the coconut water (Shenkin, 2006). Coconuts are of different sizes and varieties among which are: Malayan green dwarf, Malayan yellow dwarf, Malayan orange dwarf, West African tall coconut and the hybrid varieties (Odewale *et al.*, 2013).

Coconut fruits are said to be mature when they are 12 months and above, while the ones between 6 and 8 months are considered immature coconut fruits. The pericarp of mature coconut fruits are brownish green in colour, while the immature ones are greenish in colour. Mature coconut fruits have lesser volume of coconut water than immature coconut fruits (Jean *et al.*, 2009). The use of coconut water in aquaculture is gradually gaining attention

as observed in (Nwachi and Yuzine, 2015) who used coconut water in the hypophysation of *C. gariepinus*. This study therefore examined the efficacy and cost implication of using coconut water obtained from mature and immature coconut fruits as milt extender in induced breeding of *C. gariepinus*.

#### Materials and methods

The study was conducted in the Departmental Fish Farm of Fisheries and Wildlife Management, College of Agriculture, Ejigbo Campus which is on Latitude 7°52'28.37 and Longitude 4°18'13.76"E. Three pairs of *C. gariepinus* brood fish were selected from the Departmental fish farm based on the characteristics reported by (Olele and Tighiri, 2013). A pair of brood fish (male 1.5kg and female 1.2kg) was used at a time in this study. However, three trials were carried out. The experiment was designed to contain six treatments and three replicates. The Malayan green dwarf coconut fruits were obtained for this study. However, three (3) matured and immature coconut fruits were obtained from a coconut tree respectively. The mature ones were above 12 months while, the immature ones were 8 months old. The average weight of immature coconut fruits used for this study was 525g, while that of mature coconut fruits was 397g.

The female brood fish was induced with Ovaprim (Syndel Laboratories Ltd., Nanaimo, Canada) at 0.5mL per kg body weight to stimulate egg maturation. After latency period of 10 hours, the female fish was removed from the plastic aquarium and the eggs were stripped out into a dry bowl. The stripped eggs were then divided into each replicate such that each replicate received average of 1g of eggs which contained 700 pieces of eggs (Viveen *et al.*, 1985). One (1mL) of milt was mixed with each of the prepared extenders (immature coconut water A10 and A20; mature coconut water B10 and B20; and physiological solution C10 and C20 which is the control experiment) at two dilution ratios of 1:10 and 1:20 in volume to volume. The mixture of milt and extenders were left for 2 minutes for total contact between the eggs and the extenders, after which the eggs were evenly distributed on the spawning nets and incubated at 27°C water temperature. In the study, the number of dead eggs, number and percentage of fertilized eggs, number and percentage hatchability and percentage survival of the hatchlings were monitored. The experiment was conducted for 14 days per trial of three trials for the study.

#### Chemical composition of coconut water and 0.9 normal saline (Physiological) solution

The chemical composition of immature, mature coconut water and 0.9 normal Saline Solution were illustrated in Table 1. From the Table,

it was observed that Sodium was common as the inorganic ion present in the coconut water and saline solution. The results of the proximate analysis of the coconut water revealed that both immature and mature coconut water contain protein, total lipid (fat), ash and carbohydrate. Coconut water was also realised to contain sugars such as: sucrose, glucose and fructose however, saline solution was discovered to contain no sugar, protein, and other parameters found in the coconut water. The pH value of mature coconut water was also observed to be relatively similar to that of saline solution.

#### Determination of fertilized eggs

The fertilized eggs were determined by direct visual counting of whitish and opaque eggs which were discovered between 8 and 10 hours of fertilization and were considered dead eggs. The number of bad eggs counted was subtracted from the total number of eggs fertilized. The translucent eggs which contain embryonic eyes at the time of polar cap formation (20minutes after fertilization) were considered fertilized eggs and the percentage of fertilized eggs was determined according to Florence (2012).

$$\text{Fertilized eggs} = \text{Total No of eggs incubated} - \text{Number of whitish eggs}$$

$$\% \text{ Fertilization} = \frac{\text{No of fertilized eggs}}{\text{Total No of incubated eggs}} \times 100$$

(Lawson, and Aguda, 2010)

#### Estimation of hatched eggs

The percentage of hatched eggs was determined after the eggs have been incubated for 26 hours under flow-through system. The hatched eggs dropped from the nets and clustered at the dark corners of the incubating containers. The spawning nets were removed from the plastic aquaria after all the hatchlings have dropped from the nets and swam to the bottom of the containers. The bad or un-hatched eggs which were still attached to the nets were counted to determine the number and percentage of hatchlings.

$$\begin{aligned} \text{No of hatched eggs} \\ &= \text{Total no of fertilized eggs} \\ &- \text{No of white eggs} \end{aligned}$$

$$\% \text{ Hatchability} = \frac{\text{No of hatchlings} \times 100}{\text{Total No of fertilized eggs}}$$

(Lawson, and Aguda, 2010)

#### Determination of hatchlings survival

The hatchlings which were raised under flow-through system had their yolk sacs completely absorbed on the third day of hatching and they were given external feed (De-capsulated Artemia) from the night of the second day after hatching till sixth

day after hatching. The hatchlings had their feed changed on the seventh day from artemia to 0.2 - 0.3mm, 0.3 – 0.5mm Coppens fish feed which was administered to the fry till day 14. The hatchlings were fed four (4) times daily from 08.00hr to 19.00hr and they were fed to satiation at each feeding ration. The survival of hatchlings from each treatment was determined on the 14<sup>th</sup> day of rearing and this was calculated through percentage survival.

*% Survival*

= *No of larvae at the end of experiment*

÷ *No of larvae at the beginning of experiment X 100*

(Lawson, and Aguda, 2010)

**Data analysis**

The results of number of dead eggs, fertilized eggs, percentage fertilization, percentage hatchlings and percentage survival obtained from the use of coconut water as extenders at different dilution ratios were subjected to one-way analysis of variance (ANOVA). The mean values obtained were compared at  $p < 0.05$  significant level, while the individual differences among treatments were separated using Duncan’s Multiple Range Tests.

**Results**

**Table i: Chemical composition of immature, mature coconut water and 0.9 normal saline solution**

Coconut Type	Immature Coconut fruit	Mature Coconut fruit	0.9 normal saline solution
Average weight of coconut (g)	565g	393g	
<b>Proximate</b>	<b>(g/100g)</b>	<b>(g/100g)</b>	<b>(g/100g)</b>
Water	5.82	5.55	96.10
Dry	94.18	94.45	-
Protein	0.12	0.52	-
Total Lipid (fat)	0.07	0.15	-
Ash	0.87	0.47	-
Carbohydrate	4.76	4.41	-
<b>Sugars</b>	<b>(g/100g)</b>	<b>(g/100g)</b>	<b>(g/100g)</b>
Total	5.23	3.42	-
Sucrose	0.06	0.51	-
Glucose	2.61	1.48	-
Fructose	2.55	1.43	-
<b>Inorganic ions</b>	<b>(mg/100g)</b>	<b>(mg/100g)</b>	<b>(mg/100g)</b>
Calcium (Ca)	27.35	31.64	-
Iron (Fe)	0.02	0.02	-
Magnesium (Mg)	6.40	9.44	-
Phosphorus (P)	4.66	12.77	-
Potassium (K)	203.70	257.52	-
Sodium (Na)	1.75	16.10	154
Zinc (Zn)	0.07	0.02	-
Copper (Cu)	0.01	0.03	-
Manganese (Mn)	0.12	0.08	-
Chlorine (Cl)	-	-	154
Sulphur (S)	0.58	-	-
Aluminium (Al)	0.07	0.06	-
Boron (B)	0.05	0.08	-
<b>Vitamins</b>	<b>mg/100dm<sup>3</sup></b>	<b>mg/100dm<sup>3</sup></b>	<b>mg/100dm<sup>3</sup></b>
Vitamin C	7.41	7.08	-
Thiamin (B1)	Trace	0.01	-
Riboflavin (B2)	0.01	0.01	-
<b>Lipids</b>	<b>(g/100g)</b>	<b>(g/100g)</b>	<b>(g/100g)</b>
Total	0.0733	0.1482	-
Fatty acids; total saturated	0.03	0.1	-
10:00	0.0007	0.0028	-
12:00	0.002	0.0274	-
14:00	0.0023	0.019	-
16:00	0.0219	0.032	-
17:00	0.0009	0.0016	-
18:00	0.0039	0.0108	-
20:00	0.0016	0.0033	-

Fatty acids, total monounsaturated	0.03	0.02	-
16:1	0.0011	0.0007	-
18:1	0.0194	0.015	-
20:1	0.0049	0.0019	-
22:1	0.0011	0.0023	-
Fatty acids, total polyunsaturated	0.0128	0.0054	-
18:2 n-6 undifferentiated	0.0114	0.0032	-
20:4 n-6	0.0014	0.0022	-
<b>Amino acids</b>	<b>mg/g defatted sample</b>	<b>mg/g defatted sample</b>	<b>mg/g defatted sample</b>
Alanine	1.13	3.88	-
γ-Aminobutyric acids	0.13	0.81	-
Arginine	1.60	0.76	-
Asparagine and glutamine	0.00	0.00	-
Aspartic acid	3.44	3.75	-
Cystine	0.43	0.11	-
Glycine	0.39	0.67	-
Homoserine	0.26	0.27	-
Histidine	0.66	0.58	-
Isoleucine	4.72	3.41	-
Lysine	0.22	0.21	-
Omithine	0.26	0.00	-
Pipecolic acid	0.52	0.95	-
Proline	0.64	1.06	-
Serine	0.00	0.00	-
Tyrosine	0.00	0.00	-
Tryptophan	0.20	0.33	-
Threonine	0.91	0.82	-
<b>Organic acids</b>	<b>(mg/100 DM)</b>	<b>(mg/100 DM)</b>	<b>(mg/100 DM)</b>
Tartaric	1.6	2.4	-
Malic	317	307	-
Citric	ND	24.8	-
Acetic	ND	1.3	-
pH	4.7	5.2	5.0

ND = Non detectable  
(1996)

Santoso *et al.*

**Table ii: Reproductive performance of *C. gariepinus* eggs treated with different extenders at various dilution ratios**

Parameters	TREATMENTS					
	A10	A20	B10	B20	C10	C20
No of eggs incubated	700±0.00	700±0.00	700±0.00	700±0.00	700±0.00	700±0.00
No of dead eggs	356.00±8.96 <sup>c</sup>	561.00±5.46 <sup>d</sup>	162.00±1.53 <sup>b</sup>	135.00±0.37 <sup>a</sup>	127.00±1.76 <sup>a</sup>	165.00±1.28 <sup>b</sup>
No of fertilized eggs	344.00±6.23 <sup>b</sup>	139.00±7.32 <sup>a</sup>	538.00±8.53 <sup>c</sup>	565.00±6.37 <sup>d</sup>	573.00±1.76 <sup>d</sup>	535.00±2.21 <sup>c</sup>
% fertilization	49.14±4.14 <sup>b</sup>	19.86±3.44 <sup>a</sup>	76.86±1.65 <sup>c</sup>	80.71±2.34 <sup>d</sup>	81.86±0.25 <sup>d</sup>	76.43±3.04 <sup>c</sup>
No of hatched eggs	283.00±1.17 <sup>b</sup>	27.00±4.21 <sup>a</sup>	478.00±0.37 <sup>c</sup>	505.00±2.21 <sup>d</sup>	513.00±1.89 <sup>d</sup>	475.00±1.43 <sup>c</sup>
% hatchability	82.27±1.53 <sup>b</sup>	19.42±4.23 <sup>a</sup>	88.85±0.24 <sup>c</sup>	89.38±0.32 <sup>c</sup>	89.53±0.32 <sup>c</sup>	88.79±0.54 <sup>c</sup>
% survival	80.78±3.22 <sup>b</sup>	0.00±0.00 <sup>a</sup>	80.78±0.43 <sup>c</sup>	90.93±0.21 <sup>c</sup>	66.18±2.13 <sup>b</sup>	78.86±1.10 <sup>b</sup>

**Table iii: Water quality parameters monitored during the breeding experiment**

Parameters	A10	A20	B10	B20	C10	C20
Temperature (°C)	27.08±0.03	27.10±0.01	27.09±0.01	27.16±0.02	27.10±0.01	27.12±0.02
Dissolved Oxygen (mg/L)	5.56±0.01	5.55±0.01	5.53±0.02	5.56±0.01	5.52±0.01	5.55±0.00
pH	7.60±0.02	7.77±0.01	7.68±0.01	7.54±0.02	7.80±0.01	7.72±0.02
Ammonia (mg/L)	0.13±0.01	0.13±0.01	0.13±0.01	0.13±0.02	0.15±0.01	0.15±0.02

**Table iv: Cost analysis of using coconut water and physiological solution in the artificial reproduction of *C. gariepinus***

Cost (₦)	Coconut water	Physiological solution
	₦120/Coconut fruit containing 250mL of coconut water	₦270/sachet of 0.9N saline solution containing 500mL
	₦0.48/mL	₦0.54/mL

The results of this study showed that mature coconut water used as extenders on the milt of *C. gariepinus* had significant effect ( $P < 0.05$ ) on the fertilization, hatchability and the survival rates of induced *C. gariepinus* eggs when compared to immature coconut water (Table ii). The percentage fertility of the eggs was highest in eggs mixed with physiological solutions at dilution ratio 1:10 (C10) however, the percentage fertility of eggs treated with mature coconut water diluted at ratio 1:20 (B20) were not significantly different ( $P > 0.05$ ) from those obtained in C10 which recorded the highest fertility rate. On the fertilized eggs, mature coconut water diluted at ratio 1:20 (B10) showed no significant difference ( $p > 0.05$ ) from 0.9N saline solution mixed with milt at ratio 1:20 (C20), as B10 was not significantly different from C20. A similar trend was also observed in the number of eggs hatched. The use of mature coconut water as extender at the two dilution ratios was not significantly different from the use of physiological solution on percentage hatchability. However, the use of immature coconut water as milt extender for *C. gariepinus* showed significant effect ( $p < 0.05$ ) when compared to mature coconut water and physiological solution. The results of water quality parameters monitored showed no significant difference among the extenders (Table iii). The cost analysis results of using coconut water over physiological solution showed that cost of producing 1ml of coconut water (₦0.48/ml) was significantly lower than that of the physiological solution (₦0.54/ml) as observed in Table iv.

**Discussion**

The highest number of dead eggs ( $561.00 \pm 5.46$ ) recorded in the eggs fertilized with immature coconut water diluted at ratio 1:20, could be attributed to high level of total sugar (5.23g/100g) and low level of electrolyte (Na) present in the immature coconut water (Omitogun *et al*, 2012). It could also be attributed to the low pH (4.7) of immature coconut water as this tends to be acidic which could be injurious to the eggs. It was also observed that increase in the concentration of immature coconut water had a negative effect on the fertilized eggs, fertilization rate, hatchability and the survival rate of the hatchlings. The low fertility rate recorded in the milt extender containing immature coconut water might be as a result of longer time taken for the eggs to get fertilized. Zarski, (2012) stated that eggs fertilization depends on the

electrolytes present in the physiological or activating solution.

The highest fertility and hatchability percentages recorded in the eggs mixed with matured coconut water at dilution ratio 1:20 is in agreement with the results of Muchlisin *et al.*, (2010) who reported coconut water at dilution level of 1:20 as best extender for *C. gariepinus* spermatozoa. The better results recorded in treatment B20 among the natural extenders could be attributed to the fact that extender is a buffer solution which contains inorganic compounds that determine the longevity, success or failure of spermatozoa (Omitogun *et al.*, 2012).

Lowest survival rate of the hatchlings recorded in C10 and 20 when compared to mature coconut water is similar to the findings of Dokuboba *et al.* (2018) who reported low survival rate in the hatchlings of *C. gariepinus* produced using physiological solutions of different concentrations. The results of water quality parameters monitored during the study revealed that the values obtained in each water quality parameters monitored were in the optimum range for fish production as reported by Boyd (1998). The cost analysis of using coconut water and physiological solution revealed that cost of producing coconut water was lower than that of physiological solution as shown in Table 4. This result agrees with Nwachi and Yuzine, (2015) who reported lower cost of production in coconut water over a synthetic hormone (Ovaprim) used in the artificial reproduction of *C. gariepinus* brood fish.

**Conclusion**

The study revealed that immature coconut water might not be a good milt extender for *C. gariepinus*, while, mature coconut water diluted at ratio 1mL of milt to 20mL of matured coconut water was a better extender for the *C. gariepinus* milt. It was also discovered that mixing mature coconut water with *C. gariepinus* milt at ratio 1:20 enhanced better fertilization, hatchability and hatchlings survival rate of *C. gariepinus*. Furthermore, it was observed from this study that using coconut water as milt extender in the artificial reproduction of *C. gariepinus* will be cost effective than using physiological solution.

**References**

Adewolu, M. A., Adeniji, C. A., and Adejobi, A. B. (2008). Feed utilization, growth and survival of *Clarias gariepinus* (Burchell 1822)

- fingerlings cultured under different photoperiods *Aquaculture* **283**:64–67.
- Boyd, C.E. (1998). Water quality for pond aquaculture research and development Series No. 43 International Centre for Aquaculture and Aquatic Environments Alabama Agricultural Experiment Station, Auburn University, Alabama
- Depeint, F., Bruce, W.R., Shangari, N., Mehta, R., and O'Brien, P.J. (2006). Mitochondrial function and toxicity: Role of B vitamins on the one-carbon transfer pathways. *Chem. Biol. Interact.* **2006**, 163, 113–132.
- Dokuboba, A., Akinrotimi, O. A., and Dahor, P. O. (2018). Effects of different physiological saline concentrations on the reproductive performance of *Clarias gariepinus* (Burchell, 1822); *Journal of Fisheries Science com* Vol. 12 (4): 004-008 (2018). 4 – 8p. DOI:10.21767/1307-234X.1000153.
- Fernandes, J.C.B., Neto, O.G., Rohwedder, J.J.R., and Kubota, L.T. (2000). Simultaneous determination of chloride and potassium in carbohydrate electrolyte beverages using in array of ion-selective electrodes controlled by a microcomputer. *J. Brazilian Chem. Soc.* **2000**, 11, 349–354p.
- Florence, O.N., and Harrison, T.O. (2012). Impact of stocking density on the polyculture of *Clarias gariepinus* and *Oreochromis niloticus*. *J. Agricult Sci Technol* **A2**, 1018-1023p
- George, E.F. and Sherrington, P.D. (1984). *Plant Propagation by Tissue Culture-Handbook and Directory of Commercial Laboratories*; Exegetics Ltd: Edington, UK, 1984.
- Jean, W. H., Yong, L. G., Yan, F. N., and Swee, N. T. (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules* ISSN 1420-3049 <http://mdpi.com/journal/molecules> 5144 – 5164p
- Lawson, E.O, and Aguda, A. (2010). Growth patterns. Diet composition and Reproduction in the Ten pounder, *Elops lacerta* from Olge lagoon. Iagos Nigeria. *Biol .J. N. Am*, 1(5), 974–984p. doi:10.525i/abjna 2010,1.5.974.984
- Muchlisin, Z. A., Nadiya, N., Nadiyah, W. N., Musman, M, and Siti-Azizah, M. (2010). Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822), *Aquaculture, Aquarium, Conservation and Legislation International Journal of the Bioflux Society*, Vol. 3(2) 119 – 124p
- Nwachi, O. F. and Yuzine, E. B. (2015). The use of coconut water in hypophysation of catfish (*Clarias gariepinus*) in Niger Delta Region; *Science Direct Agriculture and Agricultural Science Procedia* 00 (2015) 000-000. 1-8p
- Odewale, J.O., Okoye, M.N., Odiowaya, J.M., Ahanon, J.M. and Agho, C.I. (2013). Bunch and nut production of surviving coconut palms in lethal yellowing disease endemic area of Nigeria. *International Journal of Plant Breeding and Genetics*; Vol. 7(1): 57-64
- Olaleye, V.F. (2005). A review of reproduction and gamete management in the African catfish, *Clarias gariepinus* (Burchell 1822); *Ife Journal of Science* Vol. 7 No.1 pp. 63- 70
- Olele, N. F and Tighiri, O. H. (2013). Optimization of triploidy induction and growth performance of *Clarias anguillarias* ( African Catfish ) using cold shock. *Academic journal of Interdisciplinary studies* Vol 2, Issue 7, 189–196p.
- Omitogun, O. G., Ilori, O., Olaniyan, O., Amupitan, P., Oresanya, T., Aladele, S., Odofin, W. (2012). Cryopreservation of the Sperm of the African Catfish for the Thriving Aquaculture Industry in Nigeria, Current Frontiers in Cryopreservation, Prof. Igor Katkov (Ed.), ISBN: 978-953-51-0302-8, InTech Open Science 305-330p.
- Saat, M.; Singh, R.; Sirisinghe, R.G.; Nawawi, M. (2002). Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water. *J. Physiol. Anthropol. Appl. Human Sci.* **2002**, 21, 93–104.
- Santoso, U.; Kubo, K.; Ota, T.; Tadokoro, T.; Maekawa, A. (1996). Nutrient composition of Kopyor coconuts (*Cocos nucifera* L.). *Food Chem.* **1996**, 57, 299–304.
- Shenkin, A. The key role of micronutrients. *Clinical Nutr.* (2006). 25, 1–13.
- Viveen, W.J.A.R., Richter, C.J.J., Van Odot, P.G.W.J., Janssen, J.A.L., Huisman, E.A. (1985). Practical manual for the culture of the African catfish, *Clarias gariepinus*. Section for Research and Technology, Ministry for Development Co-operation; The Hague, Netherlands 128p
- Weiwar, C. J. (2011) "Can coconut water mimic human plasma?". *University of Florida Health Communications, Health Podcasts* RSS.N.p., 07 Nov. 2011. Web. 01 Oct. 2013. <<http://news.health.ufl.edu/2011/17811/multimedia/health-in-a-heartbeat/can-coconut-water-mimic-human-plasma/>>
- Żarski, D., Horvath, A., Kotrik, L., Targońska, K., Palińska, K., Krejszeff, S., Bokor, Z., Urbanyi, B., and Kucharczyk, D. (2012). Effect of different activating solutions on the fertilization ability of Eurasian perch, *Perca fluviatilis* L., eggs. *Journal of Applied Ichthyology* Vol. 28 Issue 6 967 – 972p