



Title	The Energy Metabolism in <i>Tilapia nilotica</i> : . Oxygen consumption at 20 and 26
Author(s)	MISHRIGI, Samir. Y.; KUBO, Tatsuro
Citation	北海道大學水産學部研究彙報, 29(2), 100-109
Issue Date	1978-06
Doc URL	http://hdl.handle.net/2115/23635
Type	bulletin (article)
File Information	29(2)_P100-109.pdf



[Instructions for use](#)

The Energy Metabolism in *Tilapia nilotica**

I. Oxygen consumption at 20 and 26°C

Samir. Y. MISHRIGI** and Tatsuro KUBO**

Abstract

The oxygen consumption of *Tilapia nilotica* ranging from 1.5 g up to 186.3 g were studied under 20°C and 26°C. The results suggest that 20°C is an extreme low temperature limit that rendered in particular big sizes at a disadvantage, hence a normal level of metabolism could only be achieved at higher temperatures. This is indicated from the gradual reduction of oxygen consumed to meet the routine metabolism requirements of those sizes at 20°C compared to the almost constant value at 26°C, and the failure of maturing and matured sizes of both sexes to consume and/or consumed very small amounts of oxygen (6.2-12.5 cc/kg/hr) to meet specific dynamic action energy requirements at that temperature.

The weight exponent " γ " as expressed by the T-line equation showed a value of 0.74; this is almost in agreement with the 0.8 stated value as constant for fresh water fishes. However, when calculated on the basis of males and females separately, this was found to be 0.54 and 0.32 respectively. This suggests a very high level of metabolism particularly for maturing and matured females and hence a controlling role of the maturity state of the fish upon energy metabolism.

Introduction

The basic principles of the thermodynamic theory behind the biological energy flow have been discussed by Brody¹⁾, Kleiber²⁾, and Phillipson³⁾. Many other workers investigated the energy metabolism of different marine and fresh water fish species, Brown⁴⁾, Basu⁵⁾, Mann⁶⁾, and Solomon and Brafield⁷⁾. However, Paloheimo and Dickie⁸⁾ laid the basis for the mathematical principles relating temperature and food energy to metabolism and body size.

While most of the work on energy metabolism has been concentrated almost on a single energy pathway, we find that the work done on fresh water fishes is almost confined to only three species, i.e. brown trout, *Salmo trutta*, the pike, *Esox lucius*, and the perch, *Perca fluviatilis*. Moreover, experiments in which energy metabolism has been studied while the fish is growing are very rare. The experiments in this study are thus meant to count for all the previous points. *Tilapia nilotica* was chosen for this study for several reasons; among these is that the fish represents an important part of the ecosystem in many fresh waters; besides, to understand the energy metabolism of a widely cultured fish such as tilapia as it

* *Sarotherodon* is the new name for the genus *Tilapia*

** Nanae Fish Culture Experimental Station, Faculty of Fisheries, Hokkaido University
(北海道大学水産学部付属七飯養魚実習施設)
(北海道大学水産学部付属七飯養魚実習施設 業績第7号)

grows and on a male or female basis is of special importance as far as this culture is concerned.

Before proceeding further, we wish to express our thanks and gratitude to professor Juro Yamada, Faculty of Fisheries, Hokkaido University, for the keen interest he continued to show concerning the progress of this work and for reading the manuscript. Thanks are also due to Mr. Shizuo Kimura, technician Nanae station, for his valuable help throughout this study.

Material and Methods

The fish used in the present experiments were obtained as a result of nursing, rearing and growing of hatchlings from three different breeders that bred in different periods in specially designated aquaria of different sizes and arrangements, including aeration systems, heaters, and biological filters. The temperature was kept constant all the time at 26°C ($\pm 1^\circ\text{C}$). Newly born hatchling were fed on a powdered mixture of pellets that contained 46% protein as a main constituent, chlorella and dry milk. The mixture was moistened by little water and heated gently to make it as a paste, which was kept in a refrigerator and given daily to the fish. Bigger fish were served pellets containing 46% protein only.

The respirometer used consisted of cylindrical tubes of different lengths and diameters which were determined in accordance to the size of the fish to be experimented. At one end of each tube a rubber stopper was fixed through which passed an inlet glass tube, while at the other end an elbow was fitted, this had a screw cover at its upper exposed side through which passed the outlet tube. These bottles were found to be easily manageable from all points of view; introduction and removal of the fish from the bottle, getting rid of any air bubbles which might have found their way to the inside of the bottle, less excitement for the fish because of adequate space availability and easy, steady and consistent flow of water resulting in a fine evenly dispersed downstream from the inlet to the outlet with no possibilities for any eddies to take place and thus interfere in the oxygen consumption measurements.

Air saturated water was allowed to circulate through the bottle after the introduction of the fish by means of a pump from a specially designed aquarium with heaters which controlled the water temperature. The rate of flow was regulated by means of two separate ways; the first was through an overflow exit, this leading again to the original aquarium by a special tube and before the water reached the oxygen bottle, and the other way was by means of a screw fitted to a silicon tube fitted in turn to the outlet of the oxygen bottle used normally to collect water samples for oxygen content determination. All the tube connections used were silicon tubes to avoid the effect of permeability of rubber tubing to oxygen, and the water flow was always adjusted to such a low value that did not produce any sort of turbulence inside the bottle.

Fish of different but successive sizes of both sexes were experimented both under unstarved and starved conditions. In each case the experiment lasted for about 24 hours and water samples were analysed every 2 to 3 hours. The oxygen bottle after the introduction of the fish was covered by a black thin polythene

sheet to prevent the fish from the effects of any external stimuli. The time of introducing the fish in the oxygen bottle was always the same, i.e. about 30 minutes after the morning meal in case of unstarved fish, and after about 20-30 hours starvation in case of starvation experiments, determined on the basis that the small sizes were observed to continue releasing fecal matter up to about 30 hours starvation while the big sizes did not release any beyond a period of about 20 hours since their last meal. This has been taken as an indication of no more energy was being spent to utilise any food material still retained by the body.

The sex and maturity state of the fish were determined depending on both the number of orifices and the degree of response to gentle pressure along the belly sides. Females have three orifices i.e. anus, oviduct, and urethra while males have two i.e. anus, and urino-genital orifice. Depending on the size of the released eggs and the thickness of the released milt the fish were classified as III, IV, or V stages of maturity.

At each instance two water samples were taken, one for the water before entering the oxygen bottle and the other for the water leaving the oxygen bottle. The oxygen content was determined by the unmodified Winkler method. By knowing the rate of flow and the difference in the oxygen content between the two samples, the oxygen consumption was calculated as the amount consumed by the fish per hour or per kilogram per hour.

While experiments with unstarved fish were to determine the routine metabolism taken as the average oxygen consumption within 24 hours, the lowest value of oxygen consumed in this case was considered as a reflection to the energy needed for both standard metabolism and specific dynamic action. However, under starved condition, the lowest oxygen consumption was taken as that needed to liberate only the standard metabolism energy requirements, hence

Table 1. *Total, standard, specific dynamic action, and routine metabolism*

Length (cm)	Weight (g)	Sex and maturity	Total metabolism	
			cc/fish/hr	cc/kg/hr
4.6	1.45	Im.	0.453	311.93
6	3.44	Im.	0.991	288.19
8.6	10.5	Im.	2.339	222.75
9.4	13	Im.	2.794	214.9
11	22.2	Im.	4.155	187.16
11.8	31.6	♀ III	6.74	213.15
13.4	42.2	♀ IV	6.57	155.56
13.9	44.4	♂ IV	6.48	145.95
13.5	46	♀ V	7.26	157.8
14	58.8	♂ IV	8.53	145.02
15.5	61	♀ V	7.52	123.28
16	67.8	♂ V	7.31	107.68
16.8	78.2	♀ oozing	9.943	127.15
17	90	♂ oozing	11.68	129.79
18	103.2	♂ oozing	12.49	121.01
19	106	♀ oozing	13.56	127.92
21.5	154	♂ oozing	17.42	113.08
23	186.3	♂ oozing	19.66	105.5

the oxygen consumed to liberate specific dynamic action energy requirements could be deduced. The total metabolism is the sum of oxygen consumed for standard metabolism, specific dynamic action and routine metabolism.

Results and Discussion

Reviewing the data of oxygen consumed for the different energy pathways, as it appears in Table 1, it shows that the nature in the decrease of oxygen consumed per kilogram per hour is maturity state dependant, accordingly, it is clear that to deal with the standard metabolism we can classify into four groups. The first group consists of fish up to 22.2 g, consuming a mean value of 96.4 cc/kg/hr of oxygen, followed by fish up to 58.8 g that consume a mean value of 75.4 cc/kg/hr, followed by fish up to 106 g which consume a mean value of 47.17 cc/kg/hr, and fish up to 186.3 g that consume a mean value of 32.06 cc/kg/hr, (Table 3). These four mean values of standard metabolism were found to be significantly different with one another at a 0.05 point, indicating that as the fish grows bigger, the energy required for maintaining the unit body weight gradually decreases. Hence, while the standard metabolism at 26°C appears to be a general weight dependant, it is more likely a maturity state dependent within their respective different size ranges, Fig-I.

On the other hand, if we consider the specific dynamic action, which reflects to a large extent the energy cost of food utilization (digestion, assimilation and excretion), we observe that based on a kilogram as a unit weight, there is a decrease in the oxygen consumption from a mean value of 39.24 cc/kg/hr in fish up to 22.2 g, to almost a constant mean value of 28.78 cc/kg/hr for the remaining sizes. When the mean values were tested for significance, only the above two

of Tilapia nilotica at 26°C as indicated from the oxygen consumption.

Standard metabolism		Specific dynamic action		Routine metabolism	
cc/fish/hr	cc/kg/hr	cc/fish/hr	cc/kg/hr	cc/fish/hr	cc/kg/hr
0.135	93.04	0.057	39.104	0.261	179.79
0.346	100.44	0.153	44.48	0.499	143.28
1.044	99.4	0.441	42	0.854	81.35
1.208	92.89	0.513	39.41	1.074	82.6
1.51	67.88	0.693	31.22	1.955	88.06
2.22	70.22	0.903	28.56	3.614	114.37
3.27	77.42	1.16	27.39	2.15	50.91
3.2	72.07	1.18	26.58	2.1	47.29
3.65	79.44	1.32	28.74	2.28	49.61
4.39	74.62	1.59	26.98	2.55	43.42
3.12	51.15	1.76	28.85	2.64	43.28
3.38	49.79	1.904	28.08	2.02	29.79
3.614	46.21	2.572	33.89	3.71	48.05
4.19	46.6	2.55	28.3	4.94	54.89
4.83	46.84	2.93	28.3	4.73	45.9
4.92	46.42	3.23	30.46	5.41	51.04
4.84	31.4	4.24	27.5	8.34	55.17
6.09	32.73	5.25	28.18	8.21	44.59

Table 2. *Total, standard, specific dynamic action, and routine metabolism*

Length (cm)	Weight (g)	Sex and maturity	Total metabolism	
			cc/fig/hr	cc/kg/hr
5.1	2	Im.	0.558	278.8
6	4.2	Im.	1.023	243.54
10	16.2	Im.	2.384	147.17
11.5	29	Im.	3.636	125.37
13.5	44.6	♀ resorption	3.861	86.57
15	50.6	♂ resorption	4.572	90.35
14.5	63.2	♀ resorption	5.719	90.48
17	75	♂ resorption	6.128	81.71
17	80	♀ resorption	6.45	80.63
18	104	♂ resorption	6.722	64.63
17.5	109	♀ resorption	7.14	65.5
18.5	118	♂ resorption	7.728	65.49
22	166	♂ resorption	9.958	59.99
21	176.2	♂ resorption	10.98	62.31

mentioned values were found to be significantly different at the 0.05 point. This shows the difference between the standard metabolism and the specific dynamic action as energy pathways; the energy spent for the specific dynamic action does not follow the same pathway as the standard metabolism, because we find that the specific dynamic action almost remains constant, while the standard metabolism continues to show a decrease per unit weight with different sizes representing different maturity states.

This however could be understood on the basis of the breeding habits of the fish, tilapia breeds 6-8 times a year, which means that after the immature stage, the fish almost needs a constant supply of energy to cope with the almost continuous development of the gonads. This might justify a constant specific dynamic action value for both maturing and matured fish.

It is also of importance to observe that based on a unit body weight, specially at the early stages of life, the fish consumed relatively a big amount of oxygen to meet the energy requirements of standard metabolism (96.4 cc/kg/hr), in comparison to the same consumed to meet specific dynamic action requirements (39.24 cc/kg/hr). Furthermore, standard metabolism energy requirements showed a consistent decrease that brought the oxygen consumption for standard metabolism in the biggest fish to a value about 70% lower compared to the smallest ones, while specific dynamic action showed only a decrease of about 25%. This illustrates the importance of adjusting standard metabolism values as a possible means to an efficient use of the available energy in a balanced way.

For a full understanding of the energy metabolism in tilapia, we should consider the third component of the total metabolism, i.e the routine metabolism. As is seen in Table 1, we can differentiate between 3 main groups depending on the amount of oxygen consumed in relation to both the size of the fish and the maturity state. These groups are: small size fish up to 3.44 g consuming a mean value of 161.54 cc/kg/hr, followed by fish up to 22.2 g that consume 84 cc/kg/hr, followed by the third group consisting of fish up to 186.3 g which consume 49.39 cc/kg/hr,

MISHRIGI and KUBO: Energy metabolism in *Tilapia*

of *Tilapia nilotica* at 20°C as indicated from the oxygen consumption.

Standard metabolism		Specific dyanmic action		Routine metabolism	
cc/fish/hr	cc/kg/hr	cc/fish/hr	cc/kg/hr	cc/fish/hr	cc/kg/hr
0.139	69.57	0.099	49.8	0.319	159.4
0.247	58.8	0.241	57.24	0.536	127.54
0.797	45.21	0.436	26.91	1.151	71.05
1.463	50.43	0.698	24.1	1.475	50.86
2.202	49.36	—	—	1.659	37.21
2.265	44.8	0.52	10.3	1.786	35.3
2.876	45.5	—	—	2.843	44.99
2.823	37.62	0.938	12.5	2.369	31.59
3.71	46.26	—	—	2.749	34.37
4.63	44.52	—	—	2.09	20.11
4.65	42.66	—	—	2.49	22.84
4.96	42.1	—	—	2.765	23.42
5.342	32.18	1.066	6.42	3.551	21.39
6.28	35.6	—	—	4.696	26.65

Table 3. Hence, though the routine metabolism seemed to follow a more or less similar pathway as the specific dynamic action, the difference came with the fact that within the small immature sizes, and unlike the specific dynamic action and even standard metabolism, routine metabolism showed two different mean values, suggesting that the smaller size showed more response to the directive effects of the environment. Under natural conditions, extra random activities are expected for very small sizes because they are always subject to predation, and such small fish normally had to depend on themselves for the first time in searching and identifying their food items.

However, the above stated correlation between standard metabolism, specific dynamic action, and routine metabolism, could be further verified by determining the relation between total metabolism (the actual oxygen consumed by each fish and not that per unit weight), and body weight as expressed by the T-line equation:

$$\log T = \log \alpha + \gamma \log w$$

where "T" is the total metabolism, " α " is the level of metabolism, " γ " is the weight exponent, and "W" is the weight of the fish.

When the above equation was applied with all the sizes studied, i.e both mature and immature, the weight exponent " γ " was found to be 0.74, while with matured males only the value dropped to 0.54, and with females to only 0.32. However, the level of metabolism " α " in the same previous equation, was found to have a value of 0.4, 0.8, and 1.3 for the whole sizes studied, maturing and matured males, and maturing and matured females respectively, (Table 5).

Hence, it is clear that in maturing and matured fish the energy metabolism is maturity state dependant more than weight dependant, and that for females in particular, the maturity state is the directive force behind energy metabolism.

Furthermore, the energy metabolism of fish acclimatized to 20°C was studied (Table 2). This showed that based on a kilogram as a unit weight, and compared to

Table 3. Mean values of oxygen consumed for standard, specific dynamic action, and routine metabolism, together with their respective weight ranges at 26°C.

Length range (cm)	Weight range (g)	Maturity state	Mean standard metabolism cc/kg/hr	Mean specific dynamic action cc/kg/hr	Mean routine metabolism cc/kg/hr
4.6-6	1.45- 3.44	Immature	—	—	161.54±11.1
4.6-11	1.45- 22.2	Immature	96.4±4.02	39.24±1.9	84 ± 3.6*
11.8-14	31.6 - 58.8	maturing	75.4±4.4	28.78±2.5	49.39 ± 6.3
16 -19	67.8 -106	matured	47.17±1.5	28.78±2.5	49.39 ± 6.3
21.5-23	154 -186.3	matured	32.06±0.94	28.78±2.5	49.39 ± 6.3

* For length range 8.6-11 cm. and weight range 10.5-22.2 g.

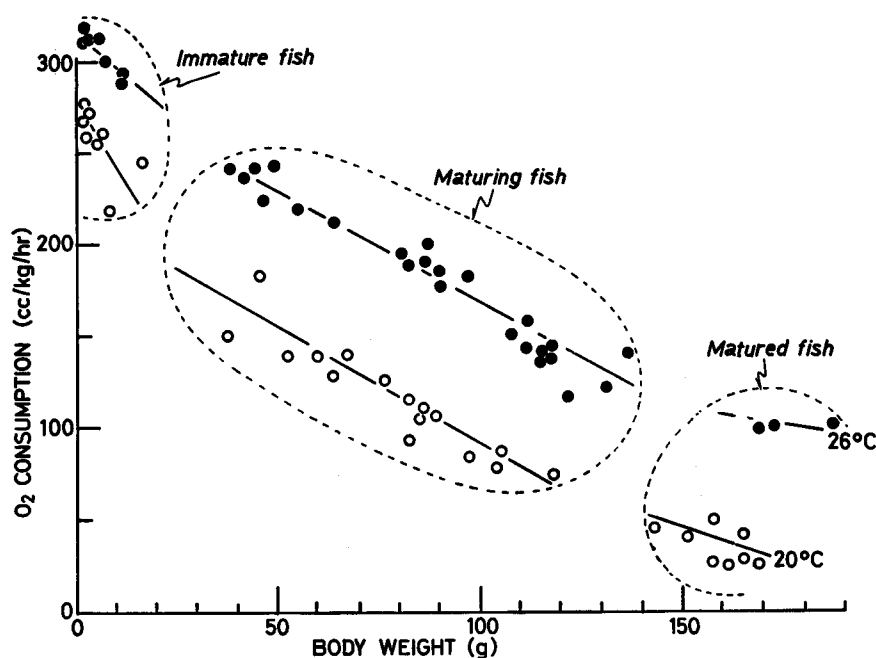


Fig. 1. Relation between oxygen consumption, weight and maturity state: The regression line for each group is drawn visually

the same at 26°C, there is a general decrease in oxygen consumption for all the involved pathways and hence the total metabolism, reflecting the general tendency of a lower level of metabolism at a lower temperature. (Fig. 1).

So, if we consider first the standard metabolism, it is also possible based on both the fish size and the maturity state to locate three significantly different mean values of oxygen consumption. These are 57 cc/kg/hr for fish ranging from 2-29 g, 44.31 cc/kg/hr for fish ranging from 44.6-118 g, and 33.9 cc/kg/hr for fish ranging from 166-176.2 g (Table 4).

Though the previous behaviour is not in complete agreement with that under

MISHRIGI and KUBN: Energy metabolism in *Tilapia*

Table 4. Mean values of oxygen consumed for standard, specific dynamic action, and routine metabolism, together with their respective weight ranges at 20°C.

Length range (cm)	Weight range (g)	Maturity state	Mean standard metabolism cc/kg/hr	Mean specific dynamic action cc/kg/hr	Mean routine metabolism cc/kg/hr
5.1- 6	2 - 4.2	Immature	—	—	143.52±16
5.1-11	2 - 29	Immature	57 ±9.4	39.51±14.8	60.96±10.2
13.5-18.5	44.6-118	maturing*	44.31±3.7	6 ±12.5**	36.69±4.5***
21 -22	166 -176.2	matured*	33.9 ±2.4	6 ±12.5**	22.89±2.45

* Resorption started ** Males only *** For fish up to 80 g.

26°C, it is still possible to conclude that the principle is the same under both temperatures. This is because, the reason why the second mean value for the maturing fish under 26°C, disappeared under 20°C, is the fact that there were no fish with maturing gonads under 20°C, and even matured fish started resorption of their gonads under this temperature.

However, that the standard metabolism under 20°C is generally weight dependant, and the degree of resorption in the gonads of maturing and matured sizes did determined the role of maturity state, is evident from the fact that fish ranging from 80-176.2 g, believed to be undergoing resorption comparatively slower than other sizes, not only followed the same pathway but even maintained the same level as at 26°C.

From the previous discussion, it is evident that *T. nilotica* could not either grow or reproduce at 20°C. This is evidenced from direct observations of the fish in the growing tank. The fish were observed not to rush and pick the falling pellets as was the case at 26°C, moreover, very few fish were seen to take few of those pellets when settled in the aquarium bottom. Evidence for resorption of gonads was clear when specimens of both males and females were cut open to check the maturity state. Ovaries were observed to be sacs filled with a yellowish fluid, and when cut open a small number of eggs which could be identified were in a non-rigid fluidy state. However, male gonads showed several patches unoccupied by any sort of tissues within each testis.

When considering the routine metabolism as shown in Table 2, we find the same phenomenon as in the case of 26°C, i.e two significantly different mean values of oxygen consumption within the immature sizes, these are 143.52 cc/kg/hr with fish up to 4.2 g and 60.96 cc/kg/hr with fish up to 29 g. However, the difference comes with bigger sizes which showed differently under 26°C, two significantly different mean values of oxygen consumption, these are 36.69 cc/kg/hr for fish up to 80 g and 22.89 cc/kg/hr for fish up to 176.2 g (Table 4). There seem to be an abnormal reduction in the daily random activity under this temperature which rendered them at a disadvantage.

As to the relation between total metabolism and weight as expressed by the T-line equation already applied in the case of 26°C, we find that based on all the sizes experimented, the weight exponent is 0.64 and the level of metabolism is 0.38. Males showed the same weight exponent and level of metabolism as the previous

Table 5. Values of both weight exponent and level of metabolism under different temperatures.

Temperature (°C)	Weight exponent (γ)			Level of metabolism (α)		
	Immature and mature fish	Males	Females	Immature and mature fish	Males	Females
20	0.64	0.64	0.71	0.38	0.38	0.33
26	0.74	0.53	0.32	0.4	0.8	1.3

ones, and females showed a weight exponent of 0.71 and a level of metabolism of 0.33, these do not seem to be significant differences from the values of 0.64 and 0.38 (Table 5), suggesting no specific values for both sexes as far as those two coefficients are concerned as was the case under 26°C.

Still when compared with values of the two coefficients under 26°C, we observe that while the weight exponent for males is higher (0.53 at 26°C and 0.64 at 20°C), the level of metabolism dropped from 0.8 at 26°C to 0.38 at 20°C. Females showed also more pronounced effects of the fish weight through a higher weight exponent (0.32 at 26°C and 0.71 at 20°C), and the level of metabolism dropped almost to the third (1.3 at 26°C and 0.33 at 20°C), i.e. under 20°C, the total metabolism is merely weight dependant.

A general conclusion for the energy metabolism in *T. nilotica* based on the present study, suggests that the two temperatures i.e 26°C and 20°C used seem to include one extreme end, that is 20°C being the lowest temperature limit below which the fish would be at a complete disadvantage, while above it the fish would gradually recover to the normal level of metabolism.

On the other hand, the energy metabolism in relation to the body weight expressed by the T-line equation, as appeared in many publications of Warren and Davis⁹⁾, Beamish and Dickie¹⁰⁾, among others, suggests that a weight exponent of about 0.8 is a constant value for all fresh water fishes. However, this value seems to fit with tilapia only when the calculation is not made on the basis of both sexes. As already shown, a value of 0.74 was found with *T. nilotica* at 26°C for all the sizes studied, including small immature and bigger maturing and matured fish of both sexes. On the basis of the two sexes separately, totally different values for the weight exponent were obtained; a 0.54 value obtained with males at 26°C did agree with that given by Ruhland and Moris¹¹⁾ for cichlids.

Hence, the constant value of about 0.8 for the weight exponent of fresh water fishes seems also to be valid for *T. nilotica*, and the possibility that both sexes do adjust their level of energy metabolism in a way to keep the after all weight exponent around the prescribed value could not be ruled out. While this might be specifically related to *T. nilotica* and also to cichlids as a reflection of the breeding behaviour, it further stresses the importance of considering the energy metabolism of each sex separately in any future study.

References

- 1) Brody, S. (1945). *Bioenergetics and growth*, Reinhold, New York.
- 2) Kleiber, M. (1961). *The fire of life*. Wiley, New York.

MISHRIGI and KUBO: Energy metabolism in *Tilapia*

- 3) Phillipson, J. (1966). *Ecological Energetics*. Arnold, London.
- 4) Brown, M.E. (1946). The growth of brown trout, *Salmo trutta*. I-Factors influencing the growth of trout fry. *J. Exp. Biol.* **22**, 118-129.
- 5) Basu, S.P. (1959). Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. *J. Fish. Res. Bd. Can.* **16**, 175-212.
- 6) Mann, K.H. (1965). Energy transformations by a population of fish in river Thames. *J. Anim. Ecol.* **34**, 253-275.
- 7) D.J. Solomon and A.E. Brafield. (1972). The energetics of feeding, metabolism and growth of perch, *Perca fluviatilis* L. *J. Anim. Ecology* **41**, 699-718.
- 8) J.E. Paloheimo and L.M. Dickie. (1965). Food and growth of fishes, I-A growth curve derived from experimental data. *J. Fish. Res. Bd. Can.* **22**, 521-542.
- 9) Charles, E. Warren and Gerald. E. Davis. (1966). Laboratory studies on the feeding, bioenergetics, and growth of fish. P. 175-214. In Shelby. D. Gerking. (ed.), *The biological basis of fresh water fish production*. 495 P. Blackwell Scientific Publications, Oxford & Edinburgh.
- 10) F.W.H. Beamish and L.M. Dickie. (1966). Metabolism and biological production in fish. P. 215-242. In Shelby. D. Gerking. (ed.), *The biological basis of fresh water fish production*. 495 P. Blackwell Scientific Publications, Oxford and Edinburgh.
- 11) Ruhland, M.L. and R.W. Morris (1971). The relation of metabolism to size and physical activity. P 7-10. In W.S. Hoar & D.J. Randall. (eds.), *Fish Physiology*. Volum VI. 559 P. Academic Press, New York & London.