African Journal of Biotechnology Vol. 10(21), pp. 4484-4493, 23 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1895
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Changes in growth, survival and digestive enzyme activities of Asian redtail catfish, *Mystus nemurus,* larvae fed on different diets

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Accepted 25 February, 2011

A study was conducted to determine the effects of different dietary treatments on the growth, survival and digestive enzyme activities of *Mystus nemurus* larvae. Newly hatched larvae were reared for 14 days in twelve 15 L glass aquaria (for growth and survival) and eight 300 L fiberglass tanks (for enzyme samples) at a stocking density of 15 larvae L^{-1}. Beginning at 2 days, the larvae were randomly assigned to *Artemia* nauplii, a microbound diet and a 50:50 combination of live food-microbound diet, while another group was unfed. All treatments were triplicated (growth and survival) or duplicated (enzyme development). The results showed that, *M. nemurus* larvae which fed on *Artemia* nauplii gave the highest survival rate (83.7%), followed by those fed on a combination diet (56.0%) and a microbound diet (26.5%). All unfed larvae did not survive beyond Day 9. *Artemia* had also given the best growth (20.4 ± 1.4 mm TL and 37.2 ± 6.0 mg wet weight) for the catfish larvae. This was followed by the combination diet (18.3 ± 0.6 mm TL and 32.6 ± 3.4 mg wet weight) and the microbound diet (11.0 ± 0.1 mm TL and 11.9 ± 0.9 mg wet weight), respectively. Pepsin began to significantly appear in *M. nemurus* larvae at 4 days old for all treatments, while chymotrypsin, trypsin and amylase were present even in the newly hatched larvae. In general, highest enzyme activities were observed among larvae which fed on a combination diet, followed by those fed on live and artificial diets, respectively. This suggested the important role of exogenous enzymes from live food in the larval digestion particularly at the early feeding stages.

Key words: *Mystus nemurus, Artemia* nauplii, larvae, microbound diet, combination diet.

INTRODUCTION

Over the last decade, the world catfish aquaculture production has increased by 6 folds (FAO, 2009). Its total production in 2008 was 2.78 million tons (worth USD 3.92 billion) compared with 541,883 tons in 1998. The catfish production in Malaysia has also jumped by 7 folds from 7158 tons in 1999 to 81,041 tons in 2009 (Anon, 2011).

In fact, catfish farming has overtaken tilapia as the top freshwater cultured fish species.

Mystus nemurus, the Asian redtail catfish or locally known as baung, is one of cultured catfishes in Malaysia. It is a popular indigenous Malaysian freshwater food fish (Khan et al., 1990). *M. nemurus* production has only become significant in 1993 (FAO, 2009) particularly in Malaysia due to the success in its artificial breeding program (Thalathiah et al., 1988) and development of grow-out feed. To date, it is only commercially cultured in Malaysia and Indonesia either semi-intensively in ponds and pens or intensively in floating cages (Abidin et al., 2006; FAO, 2009). The annual national production of *M. nemurus* in general, has steadily increased although it remains less than 1% of the total freshwater aquaculture production (FAO, 2009).

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Abbreviations: MBD, Microbound diet; **BTEE,** benzoyl tyrosine ethyl ester; **TAME,** N-tosyl-L-arginine methyl ester; **LMWP,** low molecular weight protease.

Although, *M. nemurus* fries have been routinely produced in several hatcheries, one of the major problems during its larviculture is the larval dependency on live food. At present *Artemia* nauplii are being widely used in *M. nemurus* larviculture. It has been estimated that the optimal feeding rate for *M. nemurus* is five *Artemia* mL^{-1} for first week of feeding and ten *Artemia* mL^{-1} for the second week of feeding (Kamarudin, 1999). Other than high variability in quality of nauplii, common problems of using *Artemia* are the frequent and unpredictable fluctuations in supply and price of cysts which is considered often expensive for the importing countries such as Malaysia. In addition, hatching the cysts needs extra facility, hatchery space and labor (Kamarudin et al., 1999; Verreth et al., 1992) that not only further increase the cost of fry production but also the risks associated with live food production.

The development of a specific artificial larval diet for *M. nemurus* is therefore, desirable or necessary in order to solve most of the problems stated earlier. Other than convenient, the use of artificial feed could provide consistent supply and quality of *M. nemurus* fries. However, Verreth et al. (1992) reported that most fish larvae face some problems in utilizing artificial diets. Most of the failures of larval feeds are due to the lack of information and understanding on the larval digestive capacity (Kamarudin et al., 1994), mouth morphology and feeding behavior and ecology. This study was conducted to determine the effects of no food and different diets on the growth, survival and digestive enzyme activities of developing *M. nemurus* larvae.

MATERIALS AND METHODS

In this 2 weeks study, newly hatched *M. nemurus* larvae were randomly stocked at 15 larva L^{-1} in twelve 15 L glass aquaria for determination of growth and survival and eight 300 L fiberglass tanks for monitoring the enzyme development. All larvae were obtained through induced breeding (Thalathiah et al., 1992). At the start of exogenous feeding on Day 3, the larvae were randomly subjected to four different feeding treatments: *Artemia* nauplii (Bio-Marine Brand), a 55% protein microbound diet (Kamarudin et al., 1995) and a 50:50 combination of *Artemia*-55% protein diet, while another group was not fed (unfed). Proximate compositions of live and artificial diets are shown in Table 1. All treatments were triplicated (growth and survival) or duplicated (enzyme development).

Feeding was started on Day 3 and daily done at 0800, 1200, 1600 and 2000 h. *Artemia* were given at 5 nauplii L^{-1} in the early larval stages (Day 2 to 8) and 10 nauplii L^{-1} in the later stages, while the microbound diet (MBD) was given at *ad libitum* basis. For the combination diet, the *Artemia* at a halved density were given 30 min after the MBD was delivered to ensure that MBD was utilized. All uneaten food and fecal materials were siphoned out before every morning feeding and the water volume for each tank was maintained to the original volume.

The larvae were randomly sampled at every other day. Organogenesis of *M. nemurus* larvae completes when they are 14 days old (Harmin and Somga, 1995). For enzyme study, whole larvae were sampled before the morning feeding (Baragi and Lovell, 1986) from each fiberglass tank into five 1.5 ml Eppendorf tubes (40 larvae tube-1). Excess water was removed from each tube and all samples were kept at -80°C prior the enzyme analysis. For growth study, twenty larvae from each aquarium were randomly measured for total length and body weight.

The water in each culture tank was 50% changed during the samplings. Water parameters were estimated on non-sampling days. The ranges of dissolved oxygen (D.O.) (6.2 to 7.8 ppm), temperature (26 to 30°C), pH (7.76 to 7.8) and ammonia-N (0.2 to 0.6 ppm) during the study were within the acceptable range.

Enzyme solutions were prepared by homogenizing the pooled whole larval samples in the appropriate buffer solutions (Kamarudin et al., 1999) and centrifuged at 4°C and 5000 rpm for 20 min. All enzyme solutions were analyzed within 24 h after preparation. Pepsin, chymotrypsin, trypsin and amylase were quantified in U using hemoglobin, benzoyl tyrosine ethyl ester (BTEE), N-tosyl-L-arginine methyl ester (TAME) and soluble starch as substrates, respectively (Table 2). Larval protein content was estimated using Bio-Rad protein test kit (Bradford).

All data were subjected to one way analysis of variance (ANOVA) and means differences were analyzed using Duncan's multiple range test (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

The effects of different feeding treatments on the survival and growth (Figure 1) of *M. nemurus* larvae are summarized in Table 3. None of the unfed larvae survived beyond 9 days old. The best survival and growth were achieved when the larvae were fed on live food, followed by those fed on a combination diet and artificial diet. Larval growth was more defined for body weight. After 4 days, larval body weight sharply increased with the inclusion of live food in its diet (partial or whole). All treatment means were significantly different ($P < 0.05$).

Pepsin was absent in newly hatched *M. nemurus* larvae (Figure 2). Although, traced activity was detected in 2 days old larvae, significant pepsin activity began when they reached 4 days old. In general, total pepsin activity among fed larvae increased with the larval age but the increase was slower and much lower among those fed solely on the artificial diet. In fact, pepsin activity among the later and unfed larvae was almost identical up to 6 days old. Only traced pepsin activity was observed among unfed larvae at 8 days old.

Specific pepsin activity increased sharply in larvae fed on *Artemia* and the combination diet from 4 to 6 days old and remained high. The activity among those fed solely on the artificial diet slowly increased to a peak at 12 days old.

Other digestive enzymes were present in newly hatched larvae (Figures 3, 4 and 5). Generally, total chymotrypsin activity was higher and increased with larval age when *Artemia* was solely or partially included in the larval diet. Other treatments showed a decreasing trend in total activity although, peaks were observed at 6 and 8 days old for unfed and the artificial diet. Specific

Table 1. Proximate composition of test diets.

	Nutrient (g kg^{-1} dry matter)				Gross energy (kJ g^{-1})
	Protein	Lipid	Ash	Fiber	
Artemia nauplii	553.7	167.3	59.3	4.6	235.4
Microbound	635.1	66.4	73.2	7.2	220.5

Table 2. Substrates, buffer solutions and parameters used for enzyme analyses.

Enzyme	Substrate	Buffer solution	pH	Wavelength (nm)	Temperature (°C)	Reference
Pepsin	Hemoglobin	Phenol	1.8	280	35.5	Rick and Fritsch (1974)
Chymotrypsin	BTEE	Tris	7.8	256	25	Rick (1974a)
Trypsin	TAME	Tris	8.1	247	25	Rick (1974b)
Amylase	Starch	Phosphate	6.9	564	25	Rick and Stegbauer (1974)

chymotrypsin activity for all treatments increased by 2 days old. Except for the artificial diet, specific activity increased to peaks at 4 and 6 days old for those fed solely or partially on *Artemia* and unfed larvae. Generally, the combination diet gave a higher specific activity.

All fed larvae showed increases in total trypsin activity with larval age. Generally, the highest total activity was found among those fed on a combination diet, followed by those fed on *Artemia* and artificial diet, respectively. Specific trypsin activity among fed larvae peaked at 2 days old for all treatments. Low total amylase activity was observed in catfish larvae during the yolk absorption stage (0 to 3 days old). Following the start of exogenous feeding, the activity shot up for all treatments. It generally increased with larval age for all fed larvae with the highest for those fed on the combination diet and followed by those fed solely on *Artemia* and the artificial diet, respectively.

Specific amylase activity for all treatments peaked when the larvae were 4 days old. Another peak for those fed on a combination diet was observed at 8 days old. Generally, those fed partially or solely on *Artemia* showed a higher amylase activity than those fed solely on the artificial diet.

A good quality live food is often costly and frequently varies in reliable quality and supply. On the other hand, despite many advantages, artificial larval diets often have limited success in fish larviculture. Kolkovski (2001) have suggested that, freshwater fish larvae could have a better chance to be successfully reared without a live food supplementation due to the larger size and well-developed stage of emerging or hatching larvae. This is especially true for salmonids as they have long incubation and yolk absorption periods.

The present study, however, showed that live food (*Artemia* nauplii) gave the best performance and was essential for a successful *M. nemurus* fry production. Similar observations were made on *Javanese carp*, *Barbodes gonionotus* (Kamarudin et al., 1999), African catfish, *Clarias gariepinus* (Van Damme et al., 1990), Japanese catfish, *Silurus asotus* (Hirakawa et al., 1997) and other tropical catfishes (Table 4). In fact, most studies on tropical freshwater catfish and carps show that excellent results could be achieved if live food is partially or wholly included in the larval diet.

Appelbaum and McGeer (1998) and Petkam and Moodie (2001) have shown that, an optimal larval diet could provide a better growth (than live food) for tropical catfish *C. gariepinus* and *Clarias macrocephalus,* respectively. However, the resultant survival is usually poor or significantly much lower than that of live food. Similarly, this study showed that, although *M. nemurus* larvae could survive and grow solely on an artificial larval diet, the survival and growth performances were much inferior to those fully or partially fed with live food. Eguia et al. (2000) concluded that live food organisms are required in the first and early stage feeding (up to 4 days) for a successful larviculture of *M. nemurus*. The intermediate performance of a combination of live-artificial diet in this study confirms that, live food is particularly essential in the first feeding and early larval stages of *M. nemurus* and the slightly lower lipid and energy (but slightly higher protein) in the artificial diet could not be the main causes of its poor performance. In contrast, a combination diet gives the best results for most other tropical catfish larvae (Szlaminska and Przybyl, 1986, Table 4). Kolkovski et al. (1997a) noted that, the addition of live food to a larval microdiet could significantly enhance the diet through improved growth.

Petkam and Moodie (2001) stated that, an artificial diet may not be consumed efficiently at the start of exogenous feeding of larval catfish but at later larval

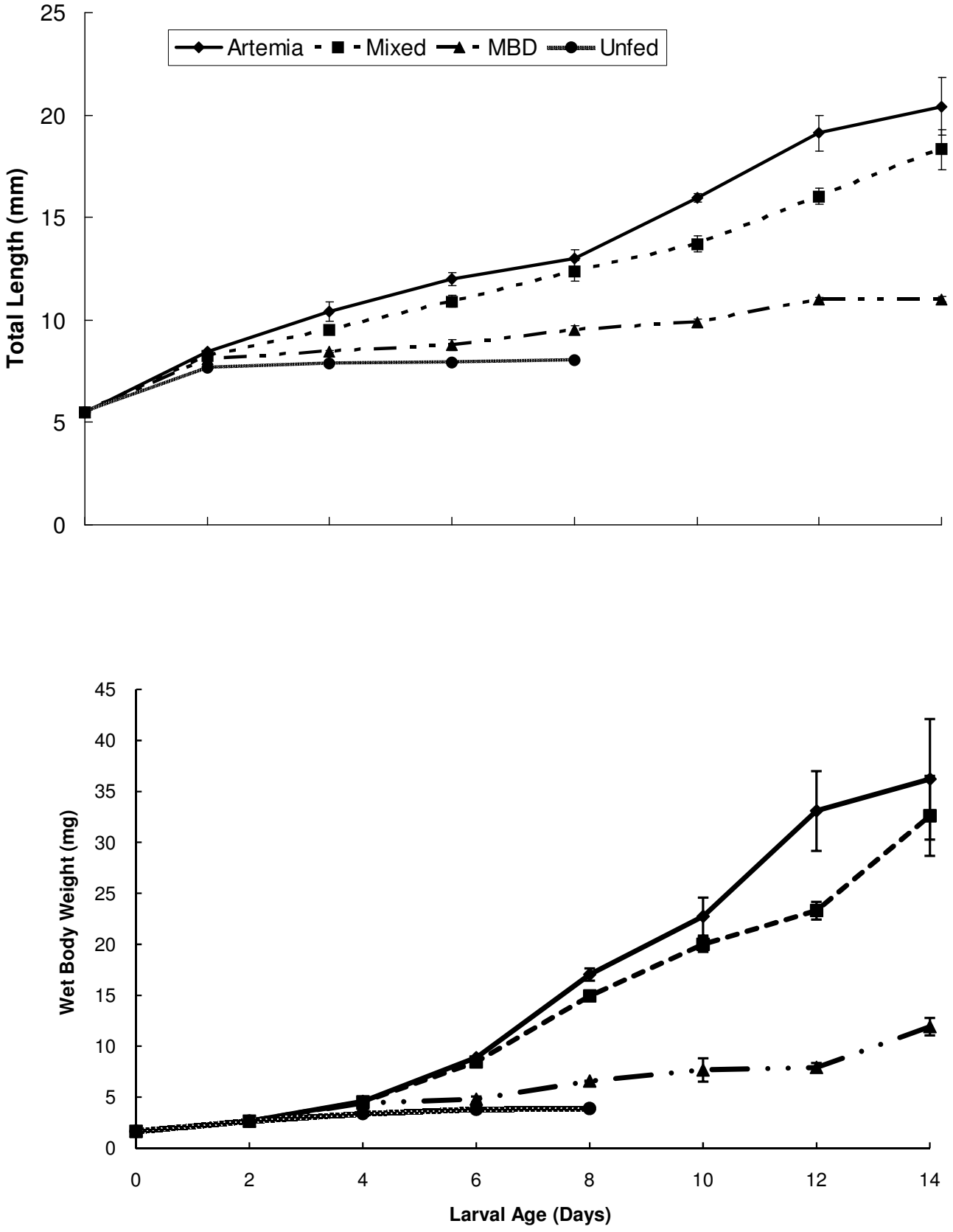

Figure 1. Growth of *M. nemurus* larvae fed on different diets.

Table 3. Mean total length, wet weight, survival and specific growth rate (SGR) of *M. nemurus* larvae fed on different diets.

Diet	Total length (mm)			Wet weight (mg)			Survival(%)	SGR(% d^{-1})
	Initial	Final	Gain	Initial	Final	Gain		
Artemia	5.51 ± 0.21	20.41 ± 1.40 [a]	14.90 ± 1.40 [a]	1.65 ± 0.01	36.19 ± 5.91 [a]	34.54 ± 5.91 [a]	83.73 ± 1.96 [a]	22.38 ± 0.04 [a]
Combination	5.51 ± 0.21	18.33 ± 0.96 [b]	12.82 ± 0.96 [b]	1.65 ± 0.01	32.60 ± 3.93 [b]	30.95 ± 3.93 [b]	56.0 ± 1.76 [b]	21.31 ± 0.08 [b]
Microbound	5.51 ± 0.21	11.02 ± 0.14 [c]	5.51 ± 0.14 [c]	1.65 ± 0.01	11.93 ± 0.86 [c]	10.28 ± 0.86 [c]	26.53 ± 2.43 [c]	14.13 ± 0.54 [c]
Unfed	5.51 ± 0.21	7.91 ± 0.18*	2.40 ± 0.18*	1.65 ± 0.01	3.9 ± 0.00*	2.35 ± 0.00*	0 ± 0 [d]	6.14 ± 0.21*

Mean ± standard deviation; *Measurements at day 8. Means within a column and followed by same alphabet are not significantly different (P > 0.05).

stage. Qin et al. (1997) suggested that, poor larval survival of an artificial diet especially at the early larval stage is probably due to the larval under-developed digestive enzyme system as enzymes are not sufficient to optimally digest non-living food. In addition, the ingestion rate of a dry larval diet in fish larvae is much lower than that of a live food (Kolkovski et al., 1993). Petkam and Moodie (2001) reported that, the performance of a paste (moist) diet among catfish larvae is better than a dry diet but is much inferior to a live food.

Understanding the ontogenetic development of the larval digestive capacity is important for the development of a specific larval feed and feeding or weaning strategy. Kuz'mina (1996) stated that, the digestive capacity in fish larvae depends on the ontogenetic development of digestive system. All digestive enzymes except pepsin were present in newly hatched *M. nemurus* larvae. Kamarudin (1999) reported that, alkaline phosphatase is also detected at this stage in the mid and posterior intestine of *M. nemurus*.

The presence of pepsin has been shown to increase digestion (Fosterck, 1992). Pepsin began to appear in traced levels in 2 days old *M. nemurus* larvae and became significant a day after the exogenous feeding started. At the same time, acid phosphatase and non-specific esterase begin to appear in the larval intestine (Kamarudin, 1999). Pepsin total activities for both artificially fed and unfed larvae were almost the same up to 8 days old when the activity dropped to a traced level for unfed larvae. Verreth et al. (1993) reported that, pepsin and acid protease appear in *C. gariepinus* larvae 4 to 5 days and 3 to 8 days after the start of exogenous feeding, respectively. Baragi and Lovell (1986) noted the appearance of pepsin in striped bass larvae at the onset of exogenous feeding (4 days old), while Kamarudin et al. (1999) could not detect any pepsin activity in *B. gonionotus* larvae.

Verreth et al. (1993) suggested that, pepsin appears and becomes involved in larval digestion when a functioning stomach has fully developed. This seemed to be also true for *M. nemurus* as its gut differentiation began when larvae are 4 to 5 days old, while its functional stomach is fully developed when larvae are 5 to 7 days old (El Hag, 2000). Kolkovski (2001) stated that, pepsin activity is also absent in most fish larvae prior the formation of gastric gland.

Total activity of all digestive enzymes in *M. nemurus* larvae generally increased with larval age. This is in agreement with reports by earlier workers (Hofer and Nasir Uddin, 1985; Kamarudin et al., 1996; Kamarudin et al., 1999; Kuz'mina, 1996). It is also interesting to note that, specific trypsin activity peaked in 2 days old larvae when traces of pepsin activity were detected. Pepsin could only digest a protein in the presence of trypsin (Uys and Hecht, 1987).

Artemia nauplii are excellent and most widely used first food for most freshwater and marine fish species. Other than providing some essential micronutrients, *Artemia* may contribute to an activation of zymogens or digestive hormones (Petkam and Moodie, 2001) or may increase larval endogenous enzyme secretion (Pedersen and Hjelmeland, 1988). The higher pepsin activity in *M. nemurus* larvae when *Artemia* was included (partially or solely) in their diet seemed to support this theory. The inclusion of *Artemia* appeared to enhance the larval pepsin secretion. Although, Dabrowski and Glogowski (1977) have reported the presence of pepsin-like protease (using a non-specific pepsin substrate) in several crustacean live foods including *Artemia*, crustaceans are known to contain low molecular weight protease (LMWP) rather than pepsin (Galgani and Nagayama, 1987; Zwilling and Neurath, 1981). Therefore, the higher pepsin activity in larvae could not be directly contributed by exogenous pepsin of *Artemia* nauplii. In a later study, a dietary pepsin supplementation seemed to neither

Table 4. Performances of live and artificial diets in relation to hatching size for several tropical freshwater fishes.

Species	Mean hatching size		Diet performance	Reference
	TL (mm)	BW (mg)		
M. nemurus	5.51	1.65	Survival: Microdiet << Combination << Live food Growth: Microdiet << Combination < Live food	Present study
M. nemurus	5.98		Survival: Microdiet << Combination = Live food Growth: Microdiet << Combination ≤ Live food	Eguia et al. (2000)
C. gariepinus	6.5	2.67	Survival: Microdiet < Live food ≤ Combination Growth: Microdiet ≤ Live food << Combination	Kamarudin et al. (1996)
C. gariepinus			Survival: Not stated Growth: Microdiet << Live food	Garcia-Ortega et al. (2001)
C. gariepinus			Survival: Not stated Growth: Live food << Microdiet << Combination	Appelbaum and McGeer (1998)
C. macrocephalus		0.5-0.7	Survival: Microdiet << Live food Growth: Live food < Microdiet	Petkam and Moodie (2001)
C. macrocephalus			Survival: Microdiet << Live food < Combination Growth: Microdiet << Live food < Combination	Fermin and Bolivar (1991)
C. batrachus			Survival: Microdiet << Live food Growth: Microdiet << Live food	Alam and Mollah (1988)
C. batrachus			Survival: Microdiet << Live food Growth: Not stated	Knud-Hansen et al. (1990)
P. bocourti			Survival: Microdiet << Live food Growth: Microdiet << Live food	Hung et al. (1999)
H. longifilis	<7.0	< 2.0	Survival: Microdiet << Live food Growth: Microdiet << Live food	Kerdchuen and Legendre (1994)
A. nobilis			Survival: Microdiet<< Live food ≤ Combination Growth: Live food < Microdiet < Combination	Fermin and Recometa (1988)
B. gonionotus	3.0		Survival: Microdiet ≤ Combination « Live food Growth: Microdiet ≤ Combination ≤ Live food	Kamarudin et al. (1999)
C. striatus	< 6.5	< 0.2	Survival: Microdiet << Live ≤ Combination Growth: Microdiet << Live = Combination	Qin et al. (1997) Eguia et al. (2000)

significantly increase larval pepsin activity nor a microdiet performance in terms of growth and survival (Kamarudin, 1999).

Dabrowski (1982) stated that, most small fish do not have sufficient enzymes for digesting non-living diets. It has been suggested that exogenous enzymes from live food play an important role in assisting in the digestive process in fish or crustacean larvae (Dabrowski, 1982; Jones et al., 1993; Kamarudin et al., 1999; Kolkovski et al., 1997a). Munilla-Moran et al. (1990) estimated that, live food contributes significantly 43 to 60% protease, 78 to 88% esterase and 89 to 94% amylase into the digestive system of *Scopthalmus maximus* larvae. Kolkovski et al. (1993) further demonstrated that, the assimilation rate of a microdiet can be improved by 30% with an exogenous digestive enzyme supplementation. Nevertheless, the contribution of exogenous digestive enzymes from live food in some larval fishes has also been reported to be less than 1% by some workers (Garcia-Ortega, 2000; Kolkovski, 2001).

In the present study, the total activity of non-pepsin digestive enzymes was generally higher in larvae fed on the combination diet followed by those on *Artemia*. The enzyme activities in those fed exclusively on an artificial diet were generally much lower than the latter. The results strongly suggested that, the higher activity of these enzymes could be directly contributed by *Artemia*. Nevertheless, the role of *Artemia* stated earlier may be also involved and should not be totally dismissed for these enzymes.

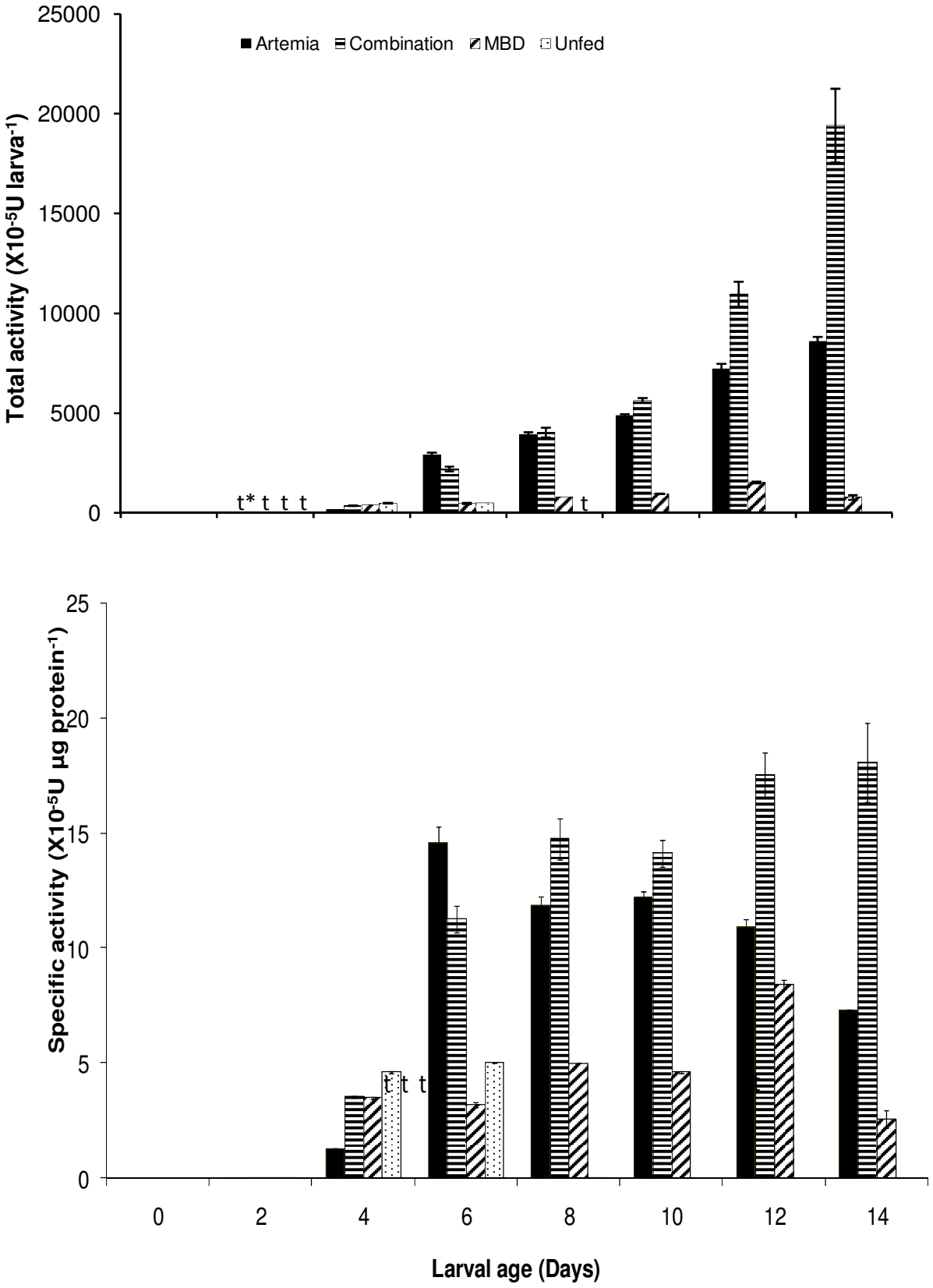

Figure 2. Pepsin activity during larval development of *M. nemurus*. * t, trace (0.0003-0.64 × 10^{-5} IU)

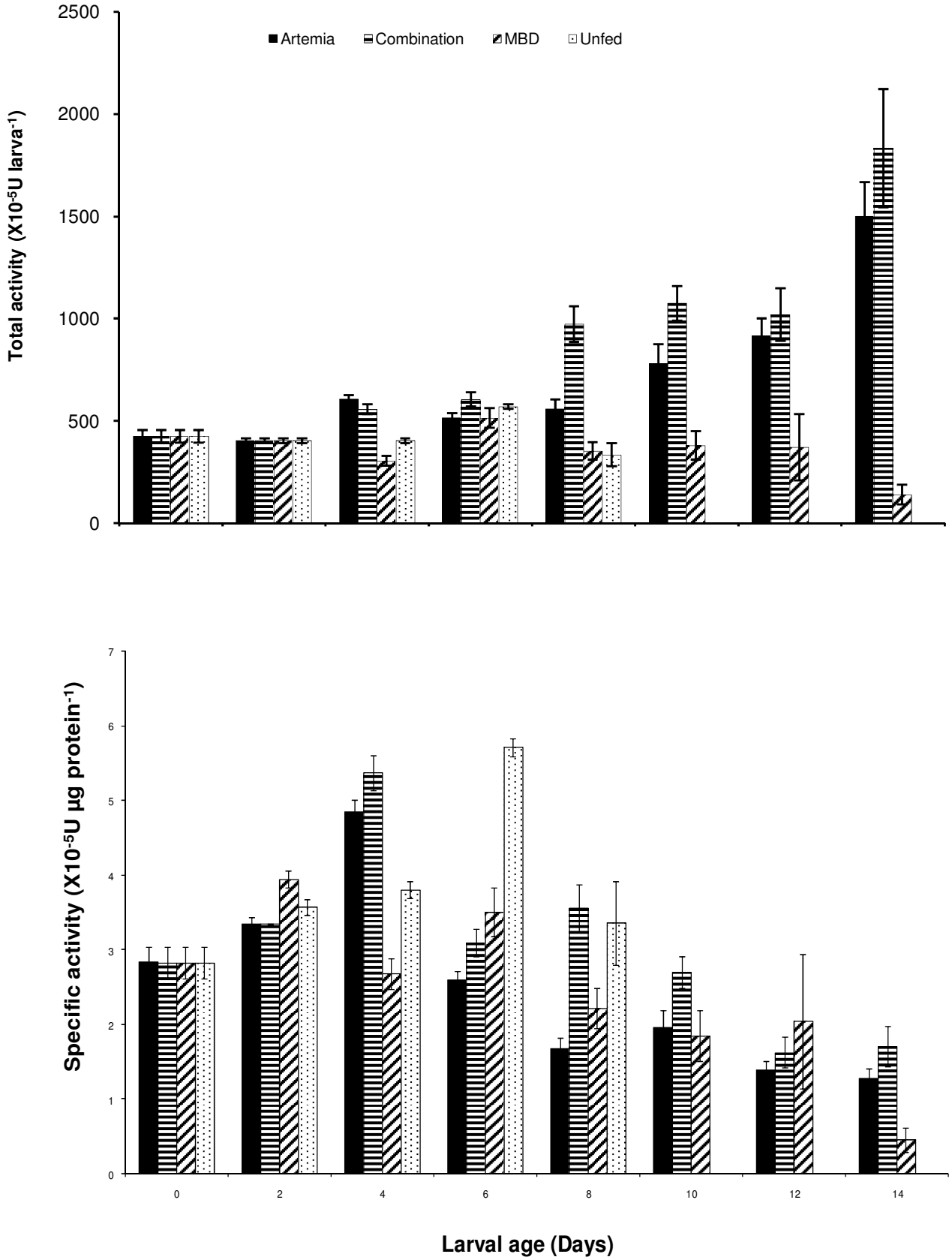

Figure 3. Chymotrypsin activity during larval development of *M. nemurus.*

Trypsin activity in fish larva is usually lower than chymotrypsin activity (Baragi and Lovell, 1986; Faulk and Holt, 2009; Faulk et al., 2007; Kamarudin et al., 1996; Kamarudin et al., 1999; Munilla-Moran et al., 1990). In the present study, trypsin activity in catfish larvae was generally higher than chymotrypsin. Chymotrypsin activity in *Artemia* nauplii is relatively low (Pan et al., 1991).

The importance of live food in the first and early stage feeding of *M. nemurus* larvae for growth and especially survival has been demonstrated from the present feeding and digestive enzyme study. Possible roles of live food in activation of zymogens or digestive hormones to enhance specific digestive enzymes and contribution of exogenous enzymes in *M. nemurus* larval digestion have been supported. Kolkovski (2001) cautioned that, the contribution of live food to the larval digestion assimilation process may also be in forms other than the direct enzyme contribution. Kolkovski et al. (1997b) have demonstrated that, both visual and chemical stimuli of live food could increase the ingestion rate of a microdiet by up to 120%. Holt (1993) suggested that, live food facilitates a better nutrient absorption as it contains approximately 75% water. In addition, Kolkovski et al. (1997a) noted that, live food enhances microdiet efficiency by promoting assimilation and deposition of dietary nutrients. An autolysis of live food could also significantly contribute to larval digestion (Hjelmeland et al., 1993; Pan et al., 1991).

Petkam and Moodie (2001) suggested that, the length of live food feeding period is crucial for a successful larviculture with high growth and survival. However, it is economically and practically desirable if larvae could be optimally weaned to an artificial diet without significantly compromising both survival and growth as early as possible. The crucial periods of live food feeding for *C. macrocephalus* and *C. gariepinus* larvae are the first 5 to 10 days and 10 to 14 days feeding, respectively (Petkam and Moodie, 2001; Verreth et al., 1993). Eguia (1998) concluded that, the best weaning scheme for *M. nemurus* larvae is a gradual wean from day 6 to day 12 after the start of exogenous feeding. The present study on the changes in larval digestive enzymes seemed to strengthen this notion.

ACKNOWLEDGEMENTS

This project was supported by the International Foundation for Science (Sweden) Grant A/2004-1 and Malaysian Government IRPA Grant 1-07-05-075.

REFERENCES

Abidin MZ, Hashim R, Chong Shu Chien A (2006). Influence of dietary protein levels on growth and egg quality in broodstock female bagrid catfish (*Mystus nemurus* Cuv. & Val.). Aquacult. Res. 37: 416-418.

Alam MS, Mollah FA (1988). Formulation of an artificial dry feed for primary nursing of catfish (*Clarias batrachus* L.) larvae. Bangladesh J. Fish. 11: 71-75.

Anon (2011). Annual Fisheries Statistics 2008 (Volume 1), Department of Fisheries, Malaysia, Kuala Lumpur, Malaysia.

Appelbaum S, McGeer JC (1998). Effect of diet and light regime on growth and survival of African catfish (*Clarias gariepinus*) larvae and early juveniles. Aquacult. Nutr. 4: 157-164.

Baragi V, Lovell RT (1986). Digestive enzyme activities in striped bass from first feeding through larval development. Trans. Am. Fish. Soc. 115: 478-484.

Dabrowski K (1982). Proteolytic enzyme activity decline in starving fish alevins and larvae. Environ. Biol. Fish. 7: 73-76.

Dabrowski K, Glogowski J (1977). Studies on the proteolytic enzymes of invertebrates constituting fish food. Hydrobiol. 52: 171-174.

Eguia R (1998). Development of an artificial diet and optimum feeding strategy for Malaysian river catfish *Mystus nemurus* (C&V) larvae. M.Sc. Thesis. Universiti Putra Malaysia. p 107.

Eguia RV, Kamarudin MS, Santiago CB (2000). Growth and survival of river catfish *Mystus nemurus* (Cuvier & Valenciennes) larvae fed isocaloric diets with different protein levels during weaning. J. Appl. Ichthyol. 16: 104-109.

El Hag GA (2000). Morphological development of mouth and gut of *Mystus nemurus* larva. Universiti Putra Malaysia. p. 100.

FAO (2010). FISHSTAT Plus ver 2.30. in: FAO Fisheries Dept. Food and Agriculture Organisation, Rome, Italy.

Faulk CK, Holt GJ (2009). Early weaning of southern flounder, *Paralichthys lethostigma*, larvae and ontogeny of selected digestive enzymes. Aquaculture, 296: 213-218.

Faulk CK, Benninghoff AD, Holt, GJ (2007). Ontegeny of the gastrointestinal tract and selected digestive enzymes in cobia *Rachycentron canadum* (L.). J.Fish Biol. 70: 567-583.

Fermin AC, Recometa RD (1988). Larval rearing of bighead carp *Aristichthys nobilis* Richardson using different types of feed and their combinations. Aquacult. Fish. Manage. 19: 283-290.

Fermin AC, Bolivar MEC (1991). Larval rearing of the Philippine freshwater catfish *Clarias macrocephalus* Gunther fed live zooplankton and artificial diet: A preliminary study. Israeli J. Aquacult. Bamid. 43: 87-94.

Fosterck CK (1992). Quality of fishmeal available to the Tasmanian salmonids industry. Australia NSW-Fisheries. pp. 221-226.

Galgani F, Nagayama F (1987). Digestive proteinases in five species of Lithodidae (Crustacea, Decapoda). Comp. Biochem. Physiol. B. Comp. Biochem. 87: 103-107.

Garcia-Ortega A, Huisman EA, Sorgeloos P, Verreth J (2001). Evaluation of protein quality in microbound starter diets made with decapsulated cysts of *Artemia* and fishmeal for fish larvae. J. World Aquacult. Soc. 32: 317-329.

Garcia-Ortega AVJSH (2000). Post-prandial protease activity in the digestive tract of African catfish *Clarias gariepinus* larvae fed decapsulated cysts of Artemia. Fish Physiol. Biochem. 22: 237-244.

Harmin SA, Somga SS (1995). Embryonic and larval development of river catfish, *Mystus nemurus* (C&V). in: Fourth Asian Fisheries Forum. Asian Fisheries Society, Beijing, China.

Hirakawa N, Takeuchi T, Sakai K, Watanabe T (1997). Feeding trial of larval Japanese catfish using live food or formulated micro-diets. J. Tokyo Univ. Fish. 83: 43-52.

Hjelmeland K, Ugelstad I, Olsen Y (1993). Proteolytic activity and post mortem autolysis in prey for marine fish larvae. in: Bernt Y, Fyhn HJ (Eds.). Physiological and Biochemical Aspects of Fish Development. University of Bergen, Bergen, Norway, pp. 229-233.

Hofer R, Nasir Uddin A (1985). Digestive processes during the development of the roach *Rutilis rutilis*. J. Fish Biol. 26: 683-690.

Holt GJ (1993). Feeding larval red drum on microparticulate diets in a closed recirculating water system. J. World Aquacult. Soc. 24: 225-230.

Hung L, Thanh T, Bui M, Cacot P, Lazard J (1999). Larval rearing of the Mekong catfish, *Pangasius bocourti* (Pangasiidae, Siluroidei): Substitution of *Artemia* nauplii with live and artificial feed. Aqua. Living Res. 12: 229-232.

Jones DA, Kamarudin MS, Le Vay L (1993). The potential for replacement of live feeds in larval culture. J. World Aquacult. Soc. 24:

199-210.
Kamarudin MS (1999). Current status of baung larval nutrition. Agro-Search. 6: 4-9.
Kamarudin MS, Longgou T, Saad CR (1995). Effect of dietary protein level on the survival, growth and feed conversion of *Clarias gariepinus* larvae. in: Lavens, P, Jaspers, E, Roelants, I (Eds.), Larvi '95 - Fish and Shellfish Larviculture Symposium. European Aquacult. Society, Gent, Belgium, pp. 259-262.
Kamarudin MS, Abu Bakar H, Saad CR (1996). Effects of live food, artificial and mixed diet on the survival, growth and digestive enzyme activities of *Clarias gariepinus* (Burchell) larvae. in: MacKinlay D, Shearer K (Eds.). Gutshop '96: Feeding Ecology and Nutrition in Fish Symposium Proceedings. American Fisheries Society, San Francisco, pp. 19-24.
Kamarudin MS, Jones DA, Le Vay L, Zainal Abidin A (1994). Ontogenetic change in digestive enzyme activity during larval development of *Macrobrachium rosenbergii*. Aquaculture, 123: 323-333.
Kamarudin MS, Mat Isa NA, Sira SS, Saad CR (1999). Effects of diets on the survival, growth and digestive enzyme activities of *Puntius gonionotus* larvae. Sc. Internat. (Lahore). 11: 91-94.
Kerdchuen N, Legendre M (1994). Larval rearing of an African catfish, *Heterobranchus longifilis*, (Teleostei, Clariidae): A comparison between natural and artificial diet. Aquat. Living Res. 7: 247-253.
Khan MS, Ambak MA, Ang KJ, Mohsin AKM (1990). Reproductive biology of tropical catfish, *Mystus nemurus* C&V, in Chenderoh Reservoir, Malaysia. Aquacult. Fish. Manage. 21: 173-179.
Knud-Hansen CF, Batterson TR, Mcnabb CD, Hadiroseyani Y, Dana D, Eidman HM (1990). Hatchery techniques for egg and fry production of *Clarias batrachus* Linnaeus. Aquaculture, 89: 9-20.
Kolkovski S (2001). Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. Aquaculture, 200: 181-201.
Kolkovski S, Tandler A, Izquierdo MS (1997a). Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. Aquaculture, 148: 313-322.
Kolkovski S, Koven W, Tandler A (1997b). The mode of action of Artemia in enhancing utilization of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture, 155: 20, 1997.
Kolkovski S, Tandler A, Kissil GW, Gertler A (1993). The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream larvae. Fish Physiol. Biochem. 10: 203-209.
Kuz'mina VV (1996). Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture, 148: 25-37.
Munilla-Moran RR, Stark JR, Barbour A (1990). The role of exogenous enzymes in digestion in cultured turbot larvae *Scophthalmus maximus* L. Aquaculture, 88: 337-350.
Pan BS, Lan CC, Hung TY (1991). Changes in Composition and Proteolytic Enzyme Activities of *Artemia* During Early Development. SO - Comp. Biochem. Physiol. A-Comp. Physiol. 100(3): 725-730.