Study of fish specificity for the infestation with glochidia Hyriopsis myersiana.

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Abstract

The analyses of several organic and inorganic elements in the plasma of different fish species on control and infested conditions with Hyriopsis myersiana were undertaken. The more suitable composition of plasma was corresponded to the fish species which produced the highest number of transformed glochidia. The infestation assay showed that glochidia H. myersiana had short transformation period of 6-12 days to reach juvenile stage. The higher recovery peak could be seen in the plasma of common carp with lower degree in nile tilapia, giant walking catfish and stripe catfish. On the other hand, the significant increase ($P<0.05$ and 0.01) at 3rd and 6th days for Pps, Pea, Hcy2, Cys, Abu, Orn and Leu could also interfere the transformation success. It is suggested that the significant decrease ($P<0.05$ and 0.01) in Mhis1, Etn, Arg, Tau, Ser, Lys, potassium, magnesium and chloride in the plasma just postinfestation could be related to the glochidia tranformation. On the contrary, significant increase ($P<0.05$ and $P<0.01$) in Sar, Ala, Pi, tryglicerides, glucose and calcium might also induce glochidia transformation. However, correlating several aspects of present data indicated that the most determinant factor is not the specific alterations in the plasma after infestation, but the specific plasma composition in different fish species at the control time.

Keywords: Glochidia; Infestation; Fish specificity; Fish plasma composition

1. Introduction

Thailand has a great potential for producing freshwater pearl on a large scale. This is because many of Thai species freshwater pearl mussels are capable of making pearls (Nagachinta et al., 1986; Panha, 1990). However, the number of animal available to support this activity is rapidly decreasing in the natural habitats. In fact, several factors are known to limit the development of glochidia, i.e., feeding by fishes, fishing in the reproductive season and the water pollution (Bauer et al., 1980; Bauer, 1988). A factor contributing to this diminishing is the natural stress caused by glochidia discharge from female followed by water contamination with bacteria, fungi and protozoa. Finally, the encystment process with many immunological reactions from the host further reduces mussels population. The early development of glochidia H. myersiana takes place within the marsupium of the female. Upon discharging from the gravid mussel, the glochidia become encysted in the fish or some amphibians (ectoparasitism) prior to transformation into early juvenile stage (Lefevre and Curtis, 1910; Seshaiya, 1941; D'Eliscu, 1972; Walker, 1981; Kraemer and Swanson, 1985; Watters and O'Dee, 1998; Haag and Warren JR, 1999). Thus, the identification of appropriate hosts for freshwater mussels has become an important measurement to conserve and manage this precious organism (Watters and O'Dee, 1998). Natural hosts have not been identified for most species of mussel, thereby, the induction of glochidial metamorphosis is more difficult. Panha (1990) reported 11 species of fish that act as host to glochidia H. myersiana. However, the glochidia which has high specificity for host become very selective in the survival process. The degree of host specificity of glochidia varies among mussel species, ranging from those which can use a wide variety of fish to those of more specific which use only a few closely related fish species (Haag and Warren, 1999).

As a result, the natural growth and transformations of glochidia are not well controlled but being directly or indirectly obliterate at different steps. The alternative uses of artificial media to bypass the parasitic stage on host have been done (Isom and Hudson, 1982; Keller and Zam, 1990; Uthaiwan et al., 2001). Anyhow, this process is only partially successful to use with every species. To find an adequate fish plasma to add in an artificial media seems to be a fundamental goal, since the culture in vitro is know to depend largely on specific organic and inorganic sources. Thus, the propose now is to simulate a natural infestation of glochidia H. myersiana into four different species of fish and analyse several parameters of fish plasma during this parasitic period as well as evaluate the best survival and transformation conditions bases on host specificity. This could lead to an ideal media composition for culture in vitro.

During glochidia H. myersiana infestation, the organic (free amino acids, protein, glucose and triglyceride) and inorganic (Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, K^{+}, S and Cl^{-}) contents of the fish plasma were measured. The survival number of juvenile posterior to infestation was also determined in order to correlate the glochidia transformation with eventual determinant of organic or inorganic elements.

2. Materials and methods

Maeklong River in Kanchanaburi Province, Thailand, is one of the natural habitats of mussel Hyriopsis myersiana. However, a controlled process has been used for rearing this mussel for more than ten years at Vajiralongkorn Reservoir to supplement for the natural deficiency or drastic disturbance in its primitive habitat. The mussels to be used in the experimental infestation were collected from this reservoir. Gravid mussel Hyriopsis myersiana with completely brown marsupia was selected for glochidiosis. The glochidia activity suitable for infestation was tested by

the observation of shell movements under light microscope (X 100). Gravid mussel was kept in a small aquarium containing 4l of dechlorinated tap water and aeration. Glochidia were released into the aquarium within 1 hr at room temperature, 27-28°C. Four fish species were selected for infestation assays, namely, the common carp (Cyprinus carprio), nile tilapia (Oreochromis niloticus), giant walking catfish (Clarias macrocephalus x C. gariepinus) and striped catfish (Pangasius pangasius) with 44.36 ±2.0 cm, 28.02±3.71, 51.17±4.72 and 42.02±4.46 cm in total length and 1425.0± 216.51 g, 409.3±164.52, 1041.67±82.5, and 674.99±223.94 g in total weight, respectively. These fish are commonly found in the natural freshwater in Thailand and are hosts for H. myersiana (Panha, 1992). However, all the experimental fish were obtained from a fish farm and have never been infested before. Each fish species was taken to the laboratory 10 days for acclamatization before infestation and were fed once daily with commercial herbivore fish food. Common carp, nile tilapia, giant walking catfish and striped catfish were anesthetized with 1,000, 1,000, 1,500 and 1,000 mg/l of quinaldine, respectively (Sado, 1985; Uthaiwan et al., 2001). Dropping infestation method was used onto the gill raker and gill filament where most topographical distribution of the parasitic glochidia were found (Kondo, 1984; Arayawatanavij, 1992). Nine anesthetized fishes per species were infested with 2662.5±129.4 of glochidia in dechlorinated water per one branchial chamber onto the gills and holding 2 minutes for successful attachment. The incidence of parasitized fish was observed in each individual fish within three days of infestation.

The first experimental group of fish was distributed in four plastic tanks, each with three fishes per species, and used for blood collection after different days (3^{rd}, 6^{th}, 12^{th}) of infestation period. The plasma analyses on 3^{rd} and 6^{th} were chosen as critical period when higher chemical blood alteration is expected while that on 12^{th}

was lower and closer to the control. The second group was with six fishes per species. After infestation, they were transfered to another tank with plastic net-cover to protect the transformed juveniles from ingestion by other fishes. During 6^{th}–12^{th} days postinfestation of the second group (six fishes per species), material from the bottom of tanks were daily siphoned through a 150 μm nylon mesh-screen. Then, the number of transformed juveniles released from infested fishes was counted under a stereomicroscope. The third group with three non-infested fishes per species in each tank were used as control. All the experimental tanks containing 700 l of dechlorinated and aerated water were utilized in different conditions.

Fish blood was collected with syringe needle no. 18 (1.2 mm in diameter and 40 mm in length) from caudal vein in the tail area of fish. The syringe was coated with sodium heparin at 1,000 unit per ml concentration. Then, the blood was centrifuged at 1,000 and 3,000 r.p.m. for 10 minutes each. The supernatant was collected and placed in a new test tube and centrifuged again at 3,000 r.p.m. for 10 minutes. Plasma portion (clear yellow solution) was separated and filtered through 0.45 μm and 0.20 μm filter paper, respectively. The fish plasma was preferably stored below –20 °C for further analyses.

The major organic and inorganic elements in fish plasma were analysed. Thirty-three free amino acids were analyzed by ion-exchange chromatography in a ninhydrin-based detection automatic system, using a standard five-lithium-buffer system (LMB 4151 Alpha Plus® Amino Acid Analyzer) designed for physiological fluid analysis, with L-norleucine as an internal standard. Light absorbance was measured at 570 and 440 nm to evaluate the amount of hydroxyproline and proline. Protein content were measured using Biuret method (Doumas et al., 1957), triglyceride using Enzymatic method (Fossati and Prenchip., 1982; McGowax et al.,

1983), glucose using Enzymatic method (Trinder,1969) and ions (Ca^{++}, Cu^{++}, Cl^{-}, Mg^{++}, Mn^{++}, Na^{+}, K^{+}, Cl^{-} and S) on High Performance Energy Dispersive X-ray Fluorescence Spectrometer (Oxford ED^{2000} model) while the osmolality measurements was measured using freezing point osmometer (SLAMED 800cl model).

The precentage of juvenile transformation, organic and inorganic contents, osmolality in the plasma collected from control and infested fishes of different species were analyzed using ANOVA and Duncan New's Multiple Range Test.

3. Results

The incidence and survival percentage of common carp, nile tilapia, giant walking catfish and striped catfish was found to be 100. Parasitic glochidia infesting to all fish species could be transformed into juvenile. Most juvenile recovery for all fish species peaked at 7-10 days after infestation except striped catfish was found to be at 8-10 days (Fig. 1). The total number of Hyriopsis myersiana juvenile during 6th–12th days postinfestation was found to be 844 in common carp while in nile tilapia, giant walking catfish and striped catfish were 587, 503 and 107, respectively.

Free amino acid content in fish plasma of infested common carp, nile tilapia, giant walking catfish and striped catfish are shown in Table 1 for three infestation steps (3^{rd}, 6^{th}, 12^{th} days). The low concentrations at the minimum of 0-0.40±1.1µmol/l for His and at maximum of 397.93±203.5 µmol/l for Gly with non-significant difference ($P>0.05$) could be observed (Table 1). Other free amino acids, namely, Hyl, Bala, Mhis3, Cysta, Cit, Hyp, Met, Tyr, Phe, Ile, Asn, Aad, Asx, Glx, Thr, Pro and Val were found in subsequent order with non-significant difference ($P>0.05$) (Table 1). Non-significant values were also observed for osmolarity and inorganic

compounds of Cu^{2+}, Mn^{2+}, P, S, and Na^{+} in the range of 0.15±0.52 mg/g (Cu^{2+}) and 2658.15±544.01 mg/g (Na^{+}).

Other free amino acids as Mhis1, Etn, Arg, Tau, Ser and Lys were presented at higher values in the control groups within the range of 4.33±1.6 µmol/l (Mhis1) and 221.20±96.8 µmol/l (Lys), with highly significant difference ($P<0.01$) (Table 1). The same characteristic was found for inorganic elements as K^{+} (247.09±80.99 mg/g), Mg^{2+} (356.34±116.65 mg/g) and Cl^{2+} (3950.24±454.52 mg/g). These amino acids and inorganic compounds reached lower significant values during the infestation period (Table 1). On the contrary, Sar, Ala and Pi showed an increase, with a highly significant differences ($P<0.01$), on the last day (12^{th}) within the range of 11.17±20.4 and 460.75±39.7 µmol/l, respectively. Triglyceride (280.51±124.02 mg/dl), glucose (245.05±81.79 mg/dl) and calcium (197.21±73.01 mg/g) also increased significantly at the end of infestation. Amino acid analysis exhibited a significant peaks ($P<0.05$ and 0.01) on the 3rd or 6th days for Pps, Pea, Hcy2, Cys2, Abu, Orn and Leu, while specific ranges of 10.0±6.6 and 216.58±94.6 µmol/l were found for Pps and Leu, respectively (Table 1). Proteins showed the same characteristic on 3^{rd} day reaching significant maximum at 3.39±0.61.

The relative variations in free amino acid contents between the total average of infestations days ($3^{rd} + 6^{th} + 12^{th}$) and the respective control per species showed only few with significant ($P<0.05$ and 0.01) change in the plasma composition (Table 2). The concentration differences between the control and infested fishes were: Abu and Aad in common carp; Cit, triglyceride and magnesium in Nile tilapia; Abu Ile Aad, Gly, triglycerides and sulphur in giant walking fish; Abu, Met, triglycerides, sulphur and chloride in striped catfish. The rest of organic and inorganic compounds with non-significant differences ($P>0.05$) in control or infested fish species are also

presented in Table 2 from the lower concentration range of 0-6.67±11.6 mg/g for Bala, Pea, Hyl, at 2.88±0.1 mg/g for protein and at 0-0.43±0.8 mg/g for copper to the highest content of 537.75±54.6 mg/g for Ala, 381.16±38.1 mg/g for triglycerides and 4545.1±86.81 mg/g for chloride.

The control values as presented for different species refer to the same group of experiments used in larvae artificial culture (Uthaiwan et al., submitted) as a parallel set up.

4. Discussion and conclusion

The short duration of parasitic phase of unionids in a fish host is generally considered (Dartnall and Walkey, 1979; Dudgeon and Morton, 1984) as a normal and obligate behavior in natural environment. Chemical or physical contact with the fish may induce larvae releasing from the mussel, at least partially in a specific way (Jokela and Palokangas, 1993). The host specificity of fishes is now known to be an important factor which could enhance glochidia infestation and metamorphosis (Haag and Warren, 1999). From the results of Isom and Hudson (1982); Keller and Zam (1990) and Uthaiwan et al. (2001) it is possible to have technical development for culturing glochidia in vitro. This would be an incentive to find an alternative method to control mussel population which has been drastically reduced by pollution. It showed that using adequate media and plasma would improve the survival and transformation percentage of these organisms. However, the complete juvenile transformation into adult stage is difficult to achieve and the information on its process is not well-acquired. This still reinforces the idea that the specificity constitutes a relevant factor for all transformation process. Early workers pointed out also that some glochidia have greater affinity for certain regions of the body or even

specific sites of organs in the host (Suydam, 1971; Hanek and Fernando, 1977). This suggests that the mechanisms controlling the morphology development in glochidia until juvenile and definitive adult ages form a complex process which implicates various delicate steps. It was confirmed by Uthaiwan et al.(submitted) for H. myersiana that fish plasma specificity is still a relevant requirement, even if the artificial media is used. In effect, glochidia H. myersiana showed high percentage of survival (93%) and transformation (100%) which are easily accomplished in the plasma of common carp, while in nile tilapia, giant walking catfish and stripped, the percentages of these measurments were lower.

In the present work the plasma specificity observed in the infestation assay could present a parallel correspondence with that by Uthaiwan et al. (submitted). In fact, the number of transformed juveniles after infestation showed the same order of specificity as common carp, nile tilapia, giant walking catfish and stripped catfish. It means that the common carp was more available for in vitro culture of H. myersiana while the striped catfish was less as also showed in the delay of peak. The present results showed higher specificity effect when compared to the experimental results of Waller and Holland-Bartels (1988). In fact, there was a shorter peak period of 7-10 days for accomplishing the larvae transformation of H. myersiana while the peak period for Lampsilis higginsi was at 18-24 days with different fishes. However, there was a still significant reduction of glochidia number injected into the gills, since only a small fraction of transformed juveniles of H. myersiana were obtained in all experimental conditions. This suggested that an effective infestation was not undertaken and/or that the transformation of infested number was not completely successful. Such drastic decreasing is probably related to strong selective factors like

the composition and texture of connective tissue and blood of different fish species and their respective degree of immunity.

An ultimate goal was to determine if any specific amino acids or other organic and inorganic compounds in the plasma of infested fish could influence the high or low efficiency on juvenile transformation of H. myersiana. This could give us some idea about the best conditions for in vitro culture in order to induce higher juvenile survival and immune resistance while reaching the adult stage. The non-significant composition of His-Gly suggested the weak effect of these amino acids on juvenile transformation. On the contrary, the significant variations of Mhis1-Lys associated to potassium, sodium and chloride with highest contents at control (zero time), Sar, Ala, Pi, triglycerides, glucose and calcium with highest value at the end of infestation (12 days) and of Pps-Leu and protein with higher contents during 3-6 days suggested some specific influence on glochidia transformation. However, these significant differences were not consistent with those observed in the free amino acid contents from infested fish species if compared with control conditions. In fact, it was found to be Abu and Aad for common carp, Cit, triglycerides and magnesium for nile tilapia, Abu, Ile, Aad, Gly, triglycerides and sulphur for giant walking catfish and Abu, Met, triglycerides, sulphur and chloride for striped catfish.

The results also showed that the significant variations of specific organic and inorganic elements during the infestation period with all fishes might at least explain partial trend that influence the development of glochidia transformation. Although, an individual (per species) analyses of infestation effect seems to mask or dilute the specificity of the first group, this inconsistency indicated that these amino acid changes during infestation are not relevant to determine any specific behavior of different fish plasma. On the other hand, the equivalent and parallel efficiency of

control plasma in vitro culture as showed by Uthaiwan et al. (submitted) makes us believe that the difference among several fish plasma in control are enough to determine its efficiency. It means that there is no need to have any specific alteration during infestation in order to induce higher transformation. Thus, it is relevant to point out that the amino acid contents then measured by Uthaiwan et al.(submitted) for the group Cit, Glx, Leu, Pro, Thr and Ala may effectively constitute the most fundamental elements for the success of glochidia survival and transformation.

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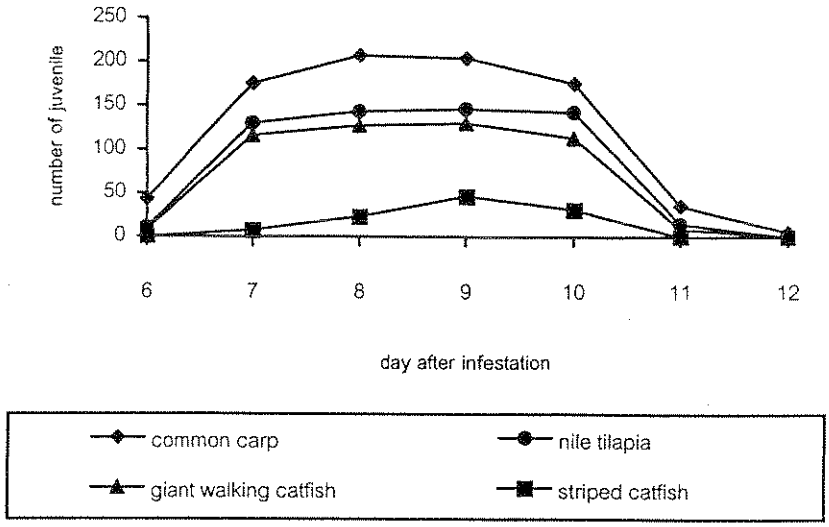

Figure 1. Recovery number of juveniles Hyriopsis myersiana during 6th–12th days after infested six fishes per species with 2,662.5±129.4 glochidia in each branchial chamber of common carp, nile tilapia, walking catfish and striped catfish.

Table 1 –The organic and inorganic elements measured in the four fish plasma species in control and during infestation periods. The values of all elements correspond to the average in all steps of plasma composition in common carp, nile tilapia, giant walking catfish and striped catfish. The elements are organized into several groups depending on the significant and non-significant differences and concentration order. The number of samples used for N represents statistic calculations.

Organic/ Inorganic elements	Time infection (days)				
	Control *	3	6	12	Sig.
Amino acids (µmol/l)					
His	0.40±1.1	0	0.18±0.6	0	ns
Hyl	0.40±1.1	1.00±0	0.18±0.6	0	ns
Bala	1.33±5.2	0	0	5.00±11.8	ns
Mhis3	8.07±9.2	8.83±11.1	6.82±9.0	8.75±10.3	ns
Cysta	10.47±19.6	25.75±51.5	8.73±28.9	11.92±21.1	ns
Cit	15.53±14.4	27.58±47.2	11.00±12.2	9.75±12.9	ns
Hyp	26.60±18.4	28.58±19.14	23.45±8.6	19.42±11.6	ns
Aad	54.93±116.9	109.42±213.8	115.55±314.3	130.83±305.5	ns
Met	55.07±30.5	40.50±19.6	45.18±23.9	59.92±28.9	ns
Tyr	69.53±20.8	61.08±43.4	52.45±12.1	60.17±31.2	ns
Phe	76.07±17.1	68.33±18.6	66.64±11.5	81.92±64.8	ns
Ile	96.33±27.9	105.50±44.46	111.36±51.0	77.92±40.4	ns
Glx	108.40±66.3	144.92±57.9	82.73±36.6	116.0±53.1	ns
Asn	113.60±92.2	93.92±55.7	82.36±64.2	62.17±38.1	ns
Asx	137.13±90.5	114.25±55.8	94.8±70.3	86.67±42.2	ns
Thr	152.60±134.4	115.75±56.1	82.18±51.8	117.08±83.4	ns
Pro	155.80±169.3	163.25±263.5	103.64±126.3	83.58±94.7	ns
Val	166.87±49.4	149.67±61.5	160.91±39.4	137.92±58.9	ns
Gly	397.93±203.5	340.92±142.5	305.64±173.9	373.33±238.5	ns
Mhis1	$4.33±1.6^{a}$	$2.92±3.2^{ab}$	$1.64±2.5^{b}$	$2.92±2.6^{ab}$	*
Etn	$18.73±22.5^{a}$	0^{b}	0^{b}	$8.33±22.46^{ab}$	**
Arg	$148.33±67.4^{a}$	$70.00±50.1^{b}$	$89.45±39.7^{b}$	$88.67±53.7^{b}$	**
Tau	$301.07±144.9^{a}$	$132.33±121.1^{b}$	$201.82±56.8^{ab}$	$285.83±168.9^{a}$	**
Ser	$109.33±49.2^{a}$	$106.75±34.4^{a}$	$71.45±36.2^{b}$	$102.00±59.5^{ab}$	**
Lys	$221.20±96.8^{a}$	$120.25±49.8^{c}$	$195.91±36.4^{ab}$	$143.25±65.2^{bc}$	**
Sar	0^{b}	0^{b}	0^{b}	$11.17±20.4^{a}$	**
Ala	$329.20±141.4^{ab}$	$360.25±157.7^{b}$	$235.64±122.9^{a}$	$369.83±202.2^{b}$	**
Pi	$374.87±197.6^{a}$	0^{b}	$427.00±17.5^{a}$	$460.75±39.7^{a}$	**
Pps	$6.80±5.8^{a}$	0^{b}	$10.00±6.6^{a}$	$7.58±6.8^{a}$	**
Pea	$0.87±1.8^{ab}$	0	$10.45±8.5^{c}$	$3.92±2.4^{b}$	**
Hcy2	$1.93±3.4^{b}$	0^{b}	$12.64±24.3^{a}$	$6.3±10.3^{ab}$	**
Cys2	$7.87±6.1^{a}$	$18.75±19.1^{b}$	$4.27±3.0^{a}$	$12.75±7.2^{ab}$	**
Abu	$11.20±5.9^{a}$	$31.17±28.2^{b}$	$18.91±17.9^{ab}$	$16.33±8.7^{a}$	**
Orn	$59.00±29.7^{ab}$	$42.00±22.9^{bc}$	$71.82±32.1^{a}$	$34.00±21.7^{c}$	**
Leu	$187.27±55.5^{ab}$	$216.58±94.6^{b}$	$198.55±61.0^{ab}$	$154.50±71.9^{a}$	*
Protein(g/dl)	$2.60±0.92^{b}$	$3.39±0.61^{a}$	$3.22±0.76^{ab}$	$3.81±0.71^{a}$	**
Triglyceride(mg/dl)	$127.48±80.13^{b}$	$190.85±67.21^{b}$	$159.77±80.45^{b}$	$280.51±124.02^{a}$	**
Glucose(mg%)	$180.74±129.67^{ab}$	$169.56±47.52^{b}$	$212.48±52.03^{ab}$	$245.05±81.79^{a}$	**
Cu^{2+}	0.15±0.52	0.16±0.29	0.43±1.35	0.26±0.46	ns
Mn^{2+}	3.76±3.69	3.71±3.35	2.76±2.55	4.22±3.90	ns
P	18.62±32.66	9.20±23.50	10.25±22.20	14.60±26.93	ns
S	554.46±170.77	576.90±89.94	552.75±70.75	477.73±162.14	ns
Na^{+}	2393.73±835.89	2658.15±544.01	2277.27±793.15	2070.96±812.92	ns
Ca^{2+}	$143.63±29.30^{b}$	$168.99±33.80^{ab}$	$173.58±34.20^{ab}$	$197.21±73.01^{a}$	*
K^{+}	$247.09±80.99^{a}$	$199.00±40.45^{b}$	$209.05±60.37^{ab}$	$222.08±65.82^{ab}$	*
Mg^{2+}	$356.34±116.65^{a}$	$321.97±70.75^{a}$	$235.00±127.56^{b}$	$234.81±61.28^{b}$	**
Cl^{2+}	$3950.24±454.52^{a}$	$3851.63±542.5^{ab}$	$3706.04±526.36^{ab}$	$3606.35±395.89^{b}$	*
Osmolarity (mOsm)	340.50±52.51	342.33±39.08	337.64±39.70	334.17±52.42	ns

Remark : ns = Non-significant different
* = Significant different
** = Highly significant different

Table 2 –The organic and inorganic elements measured in the fish plasma species are represented in average for control and whole infestation period of common carp, nile tilapia, giant walking catfish and striped catfish. The elements are organized into some groups depending on the significant and non-significant differences and concentration order. The number of samples used for N represents statistic calculations.

Organic/ Inorganic elements	Adult fishes					
	Common carp$^{\Psi}$	Common carp	Sig.	Nile tilapia$^{\Psi}$	Nile tilapia	Sig.
Amino acids (μmol/l)						
Aad	5.5 ± 6.8 ^{a}	13.17 ± 7.6^{b}	**	1.33 ± 2.3	8.11 ± 12.9	ns
Abu	10.0 ± 2.2 ^{a}	8.11 ±3.3^{b}	*	5.33 ± 2.1	9.77 ± 4.1	ns
Bala	0	0	ns	0	0	ns
Hyl	0	0.22 ± 0.4	ns	1.33 ± 2.3	0.67 ± 0.7	ns
Cysta	1.0 ± 2.0	0.67 ± 0.6	ns	0	3.78 ± 6.5	ns
Pea	1.0 ± 2.0	4.00 ± 6.1	ns	1.67 ± 2.9	3.11 ± 4.0	ns
Hcy2/Gaba	2.0 ± 4.0	16.11 ± 26.2	ns	3.3 ± 5.8	1.11 ± 1.9	ns
Pps	3.5 ± 2.9	0	ns	5.33 ± 9.2	15.89 ± 22.6	ns
Mhis3	9.25 ± 5.6	5.08 ± 3.7	ns	9.0 ± 4.4	4.77 ± 4.5	ns
Cys2	9.50 ± 5.5	8.28 ± 3.5	ns	1.33 ± 1.2	10.22 ± 15.4	ns
Hyp	14.5 ± 10.7	9.72 ± 8.4	ns	13.33 ± 11.9	26.56 ± 12.4	ns
Etn	16.25 ± 11.7	5.42 ± 9.4	ns	9.67 ± 16.7	6.22 ± 5.4	ns
Mhis1	17.25 ± 23.8	8.5 ± 7.6	ns	3.67 ± 1.2	2.0 ± 1.9	ns
Cit	36.75 ± 9.6	30.03 ± 5.9	ns	8.67 ± 2.5^{b}	28.22 ± 33.0^{a}	**
Tyr	72.0 ± 18.1	53.89 ± 16.15	ns	55.33 ± 9.9	75.56 ± 27.1	ns
Orn	74.5 ± 26.2	73.94 ± 1.1	ns	21.0 ± 9.9	33.11 ± 16.8	ns
Met	82.25 ± 29.1	59.19 ± 20.4	ns	27.67 ± 14.7	27.11 ± 5.2	ns
His	83.0 ± 61.4	94.78 ± 12.0	ns	65.3 ± 24.0	63.33 ± 1.8	ns
Phe	87.75 ± 13.2	82.36 ± 5.9	ns	76.0 ± 16.4	78.0 ± 7.2	ns
Ile	120.25 ± 26.3	104.42 ± 24.4	ns	54.67 ± 4.6	59.44 ± 6.6	ns
Ser	131.75 ± 37.0	123.81 ± 11.7	ns	78.67 ± 48.05	110.22 ± 31.0	ns
Arg	182.0 ± 49.1	152.67 ± 25.6	ns	170.67 ± 130.8	135.22 ± 71.1	ns
Asx	192.25 ± 166.3	154.31 ± 32.9	ns	119.33 ± 70.8	135.44 ±42.5	ns
Glx	197.25 ± 43.0	171.08 ± 54.3	ns	61.0 ± 25.9	96.78 ± 31.1	ns
Lys	201.0 ± 147.3	192.17 ± 17.5	ns	232.0 ± 152.6	197.67 ± 93.1	ns
Val	234.5 ± 38.2	196.39 ± 37.4	ns	131.0 ± 13.9	117.89 ± 25.7	ns
Leu	253.25 ± 47.2	246.64 ± 65.6	ns	118.33 ± 17.62	129.33 ± 24.1	ns
Tau	320.0 ± 78.2	203.78 ± 124.9	ns	240.0 ± 266.9	169.67 ± 76.8	ns
Thr	331.0 ± 148.3	222.44 ± 110.02	ns	68.67 ± 13.6	94.44 ± 25.83	ns
Gly	338.0 ± 32.1	383.45 ± 47.3	ns	223.67 ± 109.7	313.67 ± 121.1	ns
Pi	345.5 ± 230.4	255.94 ± 224.9	ns	170.33 ± 295.03	211.78 ± 235.3	ns
Pro	415.25 ± 94.6	410.08 ± 128.6	ns	73.67 ± 42.1	67.78 ± 35.9	ns
Ala	537.75 ± 54.6	502.69 ± 84.8	ns	224.67 ± 62.1	275.33 ± 85.8	ns
Protein(g/dl)	3.93 ± 1.1	2.99 ± 0.29	ns	3.86 ± 0.8	3.23 ± 0.27	ns
Glucose(mg%)	248.17 ± 83.7	251.43 ± 31.49	ns	257.07 ± 107.4	145.69 ± 18.19	ns
Triglyceride(mg/dl)	347.59 ± 120.6	181.16 ± 18.06	ns	286.7 ± 33.9 ^{a}	183.17 ± 32.33^{b}	**
Inorganic elements (mg/l)						
Cu^{++}	0.33 ± 0.49	0.07 ± 0.07	ns	0.27 ± 0.46	0.08 ± 0.14	ns
P	0.53 ± 0.46	0.48 ± 0.42	ns	1.23 ± 1.07	0.78 ± 0.29	ns
Mn^{++}	3.63 ± 4.0	0.58 ± 0.07	ns	7.23 ± 4.4	0.54 ± 0.11	ns
K^{+}	166.27 ± 33.8	171.17 ± 15.73	ns	183.80 ± 70.82	214.17 ± 4.05	ns
Mg^{++}	254.17 ± 102.1	329.1 ± 47.15	ns	191.50 ± 35.1^{b}	281.21 ± 22.31^{a}	*
Ca^{++}	271.73 ± 129.7	151.5 ± 26.22	ns	173.97 ± 21.9	182.09 ± 12.41	ns
S	632.30 ± 179.3	524.99 ± 29.00	ns	531.60 ± 191.4	678.27 ± 32.91	ns
Na^{+}	2713.57 ± 479.8	2190.2 ± 438.73	ns	654.93 ± 378.12	2625.18 ± 593.6	ns
Cl^{-}	3804.23 ± 125.0	3572.5 ± 167.35	ns	3962.13 ± 484.1	4545.1 ± 86.81	ns
Osmolality (mOsm)	335.33 ± 67.3	333.22 ± 15.55	ns	365.0 ± 71.2	365.22 ± 25.32	ns

Remark : The value in the same row that have a different superscript alphabet are significantly different

ns = Non-significant different ($P > 0.05$)
* = Significant different ($P < 0.05$)
** = Highly significant different ($P < 0.01$)
$^{\Psi}$ = Control values express measurements from Uthaiwan et al. (submitted)

Table 2 – (continued)

Organic/ Inorganic elements	Adult fishes					
	Giant walking catfish $^{\Psi}$	Giant walking catfish	Sig.	Striped catfish $^{\Psi}$	Striped catfish	Sig.
Amino acids (μmol/l)						
Abu	9.67 ± 2.1 ^{b}	37.56 ± 31.9 ^{a}	*	16.6 ± 7.1 ^{b}	24.2 ± 6.7 ^{a}	*
Ile	97.3 ± 16.2 ^{b}	137.56 ±36.4 ^{a}	**	101.6 ± 12.4	109.97 ± 14.5	ns
Aad	257.33 ± 136.65 ^{b}	353.11 ± 83.5 ^{a}	**	5.2 ± 7.7	2.51 ± 2.6	ns
Gly	717.33 ± 198.1 ^{a}	442.0 ± 250.7 ^{b}	**	358.8 ± 117.3	283.6 ± 111.1	ns
Hcy2/Gaba	0	0		2.2 ± 2.5	0.73 ± 1.3	ns
Hyl	0.67 ± 1.2	0.22 ± 0.38	ns	0	0	ns
Pea	1.33 ± 2.3	5.67 ± 8.7	ns	0	1.67 ± 2.89	ns
Mhis1	2.0 ± 1.7	1.33 ±1.2	ns	5.4 ± 0.6	3.47 ± 1.7	ns
Mhis3	4.0 ± 5.3	2.89 ± 1.4	ns	9.0 ± 15.2	17.78 ± 8.0	ns
Bala	6.67 ± 11.6	2.22 ± 3.9	ns	0	0	ns
Cit	8.33 ± 5.8	7.89 ± 2.0	ns	7.0 ± 4.0	6.22 ± 1.35	ns
Etn	9.67 ± 2.1	3.22 ± 5.6	ns	31.6 ± 34.4	10.53 ± 18.2	ns
Pps	13.0 ± 5.6	7.7 ± 4.8	ns	6.6 ± 3.2	6.56 ± 5.9	ns
Cys2	14.7 ± 6.5	16.89 ± 10.8	ns	6.4 ± 3.7	5.47 ± 2.5	ns
Hyp	35.0 ± 9.5	21.22 ± 18.7	ns	39.2 ± 21.4	34.29 ±7.5	ns
Orn	40.67 ± 16.1	39.67 ± 14.5	ns	80.4 ± 15.3 ^{a}	75.91 ± 29.3	ns
Cysta	44.67 ± 20.8	55.11 ± 29.7	ns	3.8 ± 3.8 ^{b}	1.9 ± 1.9	ns
His	47.33 ± 9.3	30.44 ± 17.7	ns	96.4 ± 29.5	63.91 ± 31.3	ns
Pro	57.67 ± 23.5	28.78 ± 28.8	ns	56.4 ± 22.9	43.46 ± 12.2	ns
Phe	71.33 ± 4.9	70.78 ± 3.5	ns	69.6 ± 23.2	59.87 ± 13.9	ns
Met	80.33 ± 18.9	71.78 ± 9.4	ns	34.6 ± 6.1 ^{a}	27.09 ± 6.7 ^{b}	**
Tyr	100.0 ± 6.1	71.89 ± 25.3	ns	57.8 ± 13.2	49.71 ± 15.1	ns
Glx	122.0 ± 17.5	135.22 ±40.8	ns	57.6 ± 23.6	60.42 ± 17.0	ns
Thr	136.67 ± 29.8	131.33 ± 69.8	ns	69.8 ± 17.1	57.71 ± 12.9	ns
Asx	145.0 ± 7.0	119.44 ± 42.3	ns	99.0 ± 16.0	72.78 ± 27.2	ns
Val	147.67 ± 31.5	169.78 ±19.4	ns	145.8 ± 22.1	153.16 ± 26.3	ns
Arg	162.0 ± 26.23	83.33 ± 81.1	ns	99.80 ± 24.1	66.6 ± 28.8	ns
Ser	168.0 ± 35.5	117.22 ± 55.5	ns	74.6 ± 18.9	57.42 ± 19.0	ns
Leu	181.0 ± 26.5	238.0 ± 49.9	ns	179.6 ± 24.2	188.2 ± 22.4	ns
Lys	265.33 ± 37.3	184.56 ± 83.1	ns	204.4 ± 43.7	177.24 ± 51.7	ns
Ala	306.67 ± 13.3	263.89 ± 69.5	ns	238.6 ± 58.5	197.42 ± 43.1	ns
Tau	428.0 ± 137.9	282.89 ± 141.8	ns	246.4 ± 69.3	197.24 ± 89.5	ns
Pi	451.67 ± 8.9	298.11 ± 258.2	ns	475.0 ± 63.34	297.22 ± 259.0	ns
Protein(g/dl)	3.77 ± 0.3	3.18 ± 0.40	ns	3.67 ± 0.9	2.88 ± 0.1	ns
Glucose(mg%)	252.70 ± 113.6	202.26 ± 22.31	ns	222.27 ± 65.1	151.0 ± 4.19	ns
Triglyceride(mg/dl)	106.60 ± 8.9 ^{b}	64.99 ± 14.93 ^{a}	**	381.16 ± 38.1 ^{a}	208.13 ± 16.96 ^{b}	**
Inorganic elements (mg/g)						
Cu^{++}	0	0.06 ± 0.05	ns	0.43 ± 0.8	0	ns
P	0.84 ± 0.74	0.41 ± 0.36	ns	0	0	ns
Mn^{++}	3.73 ± 4.3	0.56 ± 0.29	ns	2.27 ± 3.2	0.37 ± 0.17	ns
Ca^{++}	182.07 ± 5.7	175.52 ± 20.55	ns	161.07 ± 23.4	139.1 ± 0.6	ns
Mg^{++}	256.0 ± 60.9	247.51 ± 44.63	ns	246.57 ± 37.9	359.92 ± 71.6	ns
K^{+}	264.77 ± 50.5	240.94 ± 5.23	ns	273.45 ± 39.4 ^{a}	247.26 ± 29.85	ns
S	400.07 ± 25.5 ^{b}	494.93 ± 56.99 ^{a}	*	346.97 ± 16.6 ^{b}	547.31 ± 20.11 ^{a}	**
Na^{+}	2403.2 ± 475.3	2618.33 ± 485.1	ns	1564.03 ± 1118.3	2338.5 ± 331.2	ns
Cl^{-}	3486.93 ± 200.7	3533.21 ± 135.16	ns	3172.10 ± 137.5 ^{b}	3693.1 ± 54.68 ^{a}	**
Osmolality (mOsm)	332.67 ± 50.6	346.89 ± 25.47	ns	303.67 ± 11.71	315.33 ± 23.59	ns

Remark : The value in the same row that have a different superscript alphabet are significantly different

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