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Abstract

Water flea *Moina macrocopa* has been evaluated for their potential to delivery antibacterial drugs, trimethoprim (TMP) and sulfamethoxazole (SMX) to freshwater fish. Bioaccumulation of both drugs in water flea was affected by drug concentration of enrichment medium and duration of enrichment. At the drug concentrations of 10-30% (w/w) the maximum drug level in *Moina* was reached at 2 and 4h of exposure for TMP (0.06-0.38 mg/ g dry weight of water flea) and SMX (0.90-1.80 mg/ g dry weight of water flea), respectively. At the drug concentrations of 40 % (w/w) the significant higher drug uptake was obtained at 6h for TMP (0.55-0.65 mg/ g dry weight of water flea) and SMX (3.20-3.70 mg/ g dry weight of water flea). A marked decrease in TMP/SMX level upon storage of the water flea in water even at half an hour was observed and the survival of the water flea after 2h is satisfactory. It was suggested that medicated water flea should be administration freshly enriched to fish.

Introduction

The current methods of dispensing drugs directly into water or to the fish feed for prophylaxis and treatment of fish disease in aquaculture have many disadvantages. For example, bath treatment is poorly effective, promotes the resistance of bacterial strains and in particular with regard to environmental impact (Brown 1989; Hekteon, Berge 1995, Hormazabal & Yndestad 1995). The medicated fish feed are unaccepted by the fish larvae that are rasied on cultured live food. To date, many efforts have been made to develop modern formulations and novel drug delivery systems for control of aquatic diseases (Lillehaug 1989; Press & Lillehaug 1995; Wong et al. 1992; Horne 1997; Lavelle et al. 1997; Shao 2001) Recently, the administration of live food supplement with antibiotics by the technique of bioencapsulation have been studied. Many antibacterial agents such as sulphonamides, tetracyclines and quinolones have been incorporated in brine shrimp, *Artemia* nauplii for delivery to seawater animals (Mohney 1990; Nelis et al. 1991; Verpraet et al.1992; Dixon et al. 1995; Duis et al. 1995; Chair et al. 1996; Touraki et al. 1999). The drug content in fish after feeding with *Artemia* verified the success of using life food as a mean of chemical carrier. . However, the use of brine shrimp is still limited due to it high cost as an import product in many countries. Moreover, they are unsuitable for cultured freshwater animals due to their short life in freshwater.

Water flea (*Moina macrocopa*), a small aquatic crustacean (Fig.1), is excellent natural food for nursing of cultured freshwater fish. They can be easily cultured in high production using *Chorella* as algal food (Tavarutmaneegul et al. 1995). Recently, we have reported the success of enrichment norfloxacin into *Moina* at optimum drug concentration and enrichment time (Wiwattanapatapee et al. 2002). In this study, the

potential of using *Moina* as an effective drug carrier of TMP/SMX, commonly used antibacterial drugs, to freshwater fish were evaluated.

Materials and Methods

Materials

Trimethoprim (TMP) and Sulfamethoxazole (SMX) were purchased from China National Chemicals, China. A1 Selco, the commercial live food enrichment was from INVE, USA, acetonitrile and methanol, HPLC grade were from Lab-Scan, Ireland, di-sodium hydrogen phosphate dihydrate was purchased from Fluka, Switzerland, phosphoric acid and triethylamine were from Merck, Germany. All other reagents were of analytical grade and they were purchased from Merck, Germany.

Experimental Animals

Moina (4-5 days old) were kindly gifts from the aquatic animal health research center, Prince of Songkhla University, Thailand. Guppy (*Poecilia reticulata*, weight 200-250 mg, length 1.5-2.5 cm) were from National Institute of Coastal Aquaculture, Songkhla, Thailand. The fish were kept in plastic tank, water temperature 26-28 °C, pH 7-7.5. They were fed with *Moina* twice daily and were allowed to adapt to these conditions for at least 5 days prior to the experiment.

Methods

Enrichment of water flea

Moina were enriched in distilled water to which commercial live food enrichment diet A1 Selco was added at a concentration of 1.2 g/L. Combinations of TMP and SMX at a ratio of 1:5 were incorporated into enrichment diet at concentrations 10, 20, 30 and 40% (w:v, oil phase). Controls consisted of water flea in the enrichment medium without the drug. The *Moina* were enriched with aeration at 26-28 °C for 8 h, at a density of 30 water fleas/ ml. At 2-h intervals, the aeration was stopped and the suspension left for 5 min to facilitate settlement of precipitated drug. The enriched *Moina* were then decanted onto a 150 mm mesh, rinsed thoroughly and seperated into two equal subsamples. Each sample weighed 300 mg (n=6). One subsample was dried at 52-55 °C until the weight was constant and placed in a desiccator prior to weighing. Drug content of the second subsample was determined by HPLC.

Sample analysis

Enriched *Moina* samples were homogenized with 3 ml of methanol using a homogeniser (V stral, X10/25, Germany) and centrifuged (Hettich Zentrifugen, Germany) at 10,000 rpm for 10 min. The supernatant was filtered through a 0.45-µm nylon membrane filter and analysed by HPLC. The liquid chromatography consisted of an LC-5000 pump (Jasco-PU-980), a gradient unit (Jasco-LG-980-025), a detector set at 275 nm (Jasco-UV-975 (UV/VIS) and an integrator (Waters, USA). The column (C-18) was a 300 x 3.9 mm, µbondapak (Waters, USA) and was eluted with acetonitrile-water (400: 1400, v/v) containing 2 ml triethylamine, pH 5.9. The flow rate was 1 ml/min, and the temperature was ambient. The contents were expressed as µg/g dry weight of water flea. Results were compared using Student's t-test under dry weight basis (Duis *et al.* 1995).

sulfamethoxazole and trimethoprim in *Artemia* nauplii were significantly decreased after 2h storage in sea water at 18 and 25 °C , or after 4h at 5 °C , and the metabolism of sulfamethoxazole were observed at 25 °C (Touraki *et al.* 1999). In the case of *Moina*, it is possible that the depletion of drug content was mainly due to the the osmoregulation in animal body since there was no metabolite of drug detected in either sample or the storage medium in these studies. More experiments are required to verify these results. The survival of the water flea after 4h was satisfactory as more than 90% was still alive. Keeping *Moina* for longer time than this led to the decrease of number of survival animals (Data not shown). The results of these studies suggest that *Moina* enriched with sulfamethoxazole and trimethoprim should be administration freshly to fish.

Conclusion

This study shows that water flea *Moina macrocopa* can be successfully enriched with sulfamethoxazole and trimethoprim at optimum drug concentration and enrichment time. However, due to the rapid depletion of the drugs from the *Moina*, the appropriate ratio of SMX/TMP and also the dosing frequency of this medicated biocarrier to fish should be intensively studied.

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Figure legends

Figure 1 Water Flea (*Moina macrocopa*)

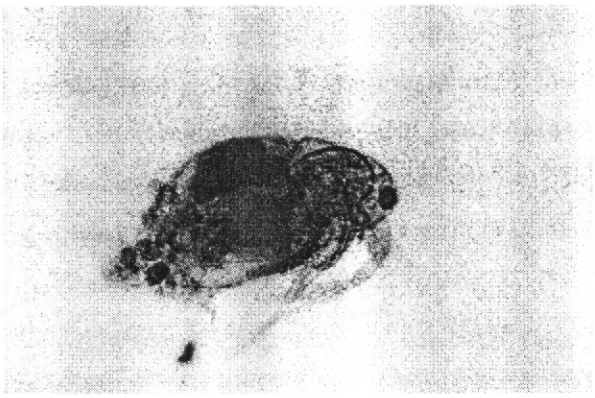
Figure 2 The level of TMP accumulated in *Moina* at different drug concentration in enrichment medium. Values represent means ± SD of six replicates.

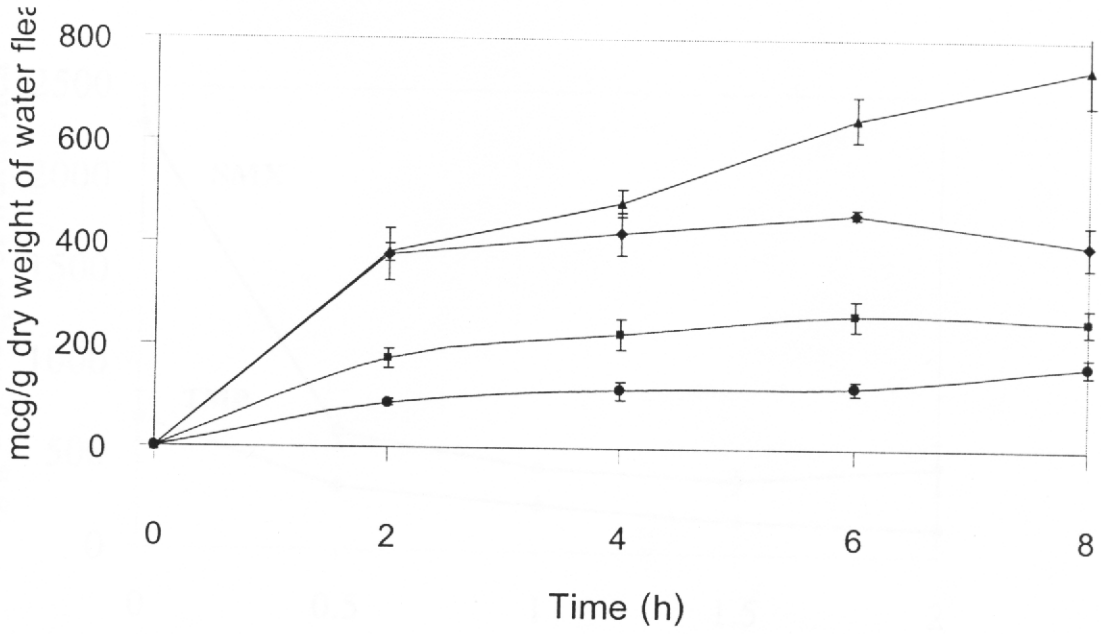
10% (●) , 20%(■), 30% (◆), 40% (▲)

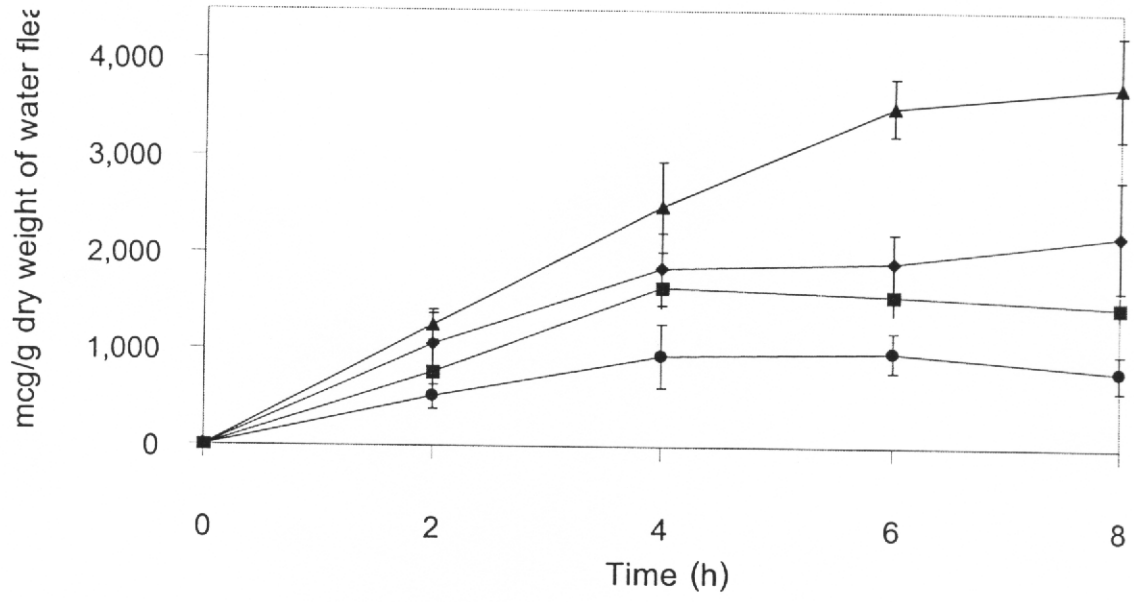
Figure 3 The level of SMX accumulated in *Moina* at different drug concentration in enrichment medium. Values represent means ± SD of six replicates.

10% (●) , 20%(■), 30% (◆), 40% (▲)

Figure 4 The level of SMX and TMP in enriched water flea during storage




mcg/g dry weight of water flea
800
600
400
200
0
0
2
4
6
8
Time (h)


mcg/g dry weight of water flea
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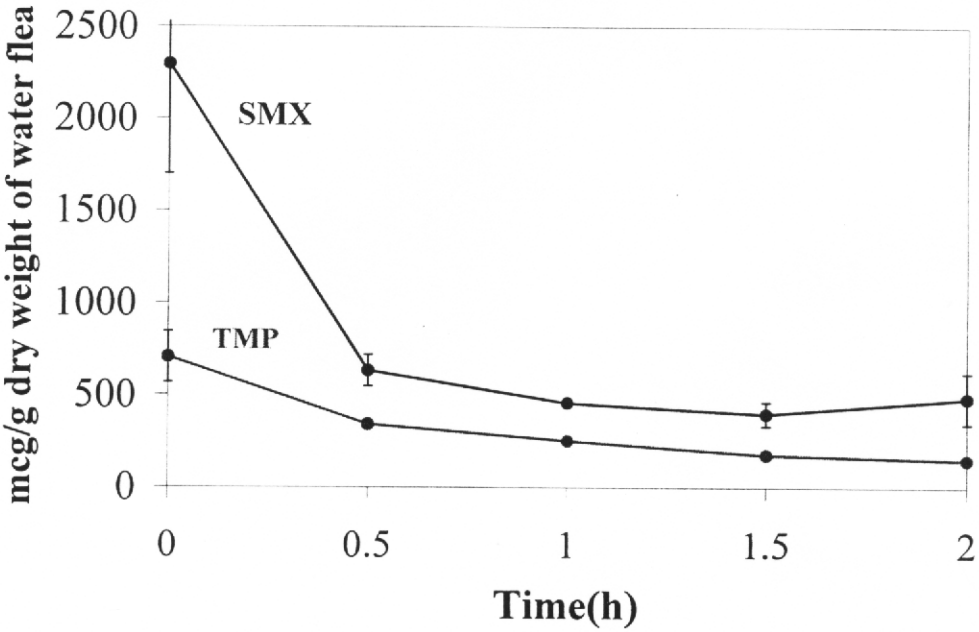
3,000
2,000
1,000
0
0
2
4
6
8
Time (h)


mcg/g dry weight of water flea
2500
2000
1500
1000
500
0
SMX
TMP
0
0.5
1
1.5
2
Time(h)