

Bioencapsulation of Praziquantel in Adult *Artemia*

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Abstract

A description of bioencapsulation of praziquantel in adult *Artemia* for 2.5 g/L, 5 g/L, and 10 g/L treatment baths is presented. Praziquantel was detected in adult brine shrimp tissue after enrichment periods of 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, and 24 hr. The assays were performed using high performance liquid chromatography. There was variable uptake by *Artemia* at all three bath treatments over time. Despite early variability, all three baths showed a terminal increase in praziquantel concentration. Highest concentration of praziquantel was seen in the initial sample (5 g/L) or the last sample (2.5 g/L and 10 g/L). The highest concentration of praziquantel at any one point was observed in the 5 g/L treatment bath at 15 minutes. Based on percentage, more praziquantel was incorporated into shrimp at the 10 g/L than either of the other treatments. Non-predictable fluctuations were seen in the concentration of praziquantel in both the treatment water and control water. Concentration of praziquantel in the control water increased in each treatment group over each of the final three time points. Neither total praziquantel in the treatment bath (shrimp and water) or the control bath were consistent among any treatment group. Survival of shrimp was not affected by concentration, but decreased over time in all treatment baths comparatively. It can be concluded that praziquantel can be successfully, but not reliably, bioencapsulated in adult *Artemia*.

Keywords: *Artemia*; Brine shrimp; Praziquantel; Bioencapsulation; Pharmacology

Introduction

The importance of aquaculture has global implications as the world population continues to grow. The success of fish culture can be jeopardized by the occurrence of infectious diseases that lead to morbidity or mortality. In order to manage aquatic animal health, the need for pharmacotherapy is unavoidable. Parasitic diseases are a major cause of disease in cultured systems and lead to significant morbidity and mortality in captive collections (Smith and Noga, 1993). Praziquantel is an anthelmintic that is used for treatment of infestations with cestodes, trematodes, and schistosomes (Plumb, 2005). It is often used in domestic animals, but is cost prohibitive in large animals and immersion dosing for aquatic systems. A cost-effective technique is needed to deliver this to animals with susceptible parasites.

When prophylactic or targeted pharmacotherapy is needed in aquatic animals, most treatment plans rely on the addition of pharmaceuticals to the feed or water. However, drug treatment methods such as these harbor risks for the environment and public health, as excess accumulation of the drug may enter waterways, thereby potentially exposing non-target individuals and species to the toxic effects of the drug. Furthermore, the individual treatment of fish using injections or oral dosing is impractical for management of disease epizootics in large collections. However, the technique of bioencapsulating a drug within a food source (live brine shrimp, *Artemia* sp.; LBS) has been used for the treatment of susceptible infectious diseases in aquatic animals with significantly less time-invested (Leger et al., 1986; Aguilar-Aguila et al., 1994). However, bioaccumulation is often performed based on anecdotal information on a few drugs. Therefore, standardization for effectively delivering a wide range of pharmacotherapeutics to fish through bioencapsulation is needed to direct future pharmacokinetic studies.

Artemia are highly palatable feed items that are non-selective filter feeders, providing an ideal transport mechanism of soluble pharmaceuticals (Dhont and Sorgeloos, 2002). Previous studies have shown that *Artemia* can accumulate pharmacologic agents, such as

antimicrobials (Cook and Rust, 2002; Gomes et al., 2007). Some of these drugs have attained therapeutic levels in fish that are fed the *Artemia* (Duis et al., 1995; Touraki et al., 1999). Currently, no studies are reported on the bioencapsulation of praziquantel in *Artemia*.

The specific objectives of this study were to: 1. determine praziquantel concentration in live brine shrimp using a bioaccumulation method; and, 2. demonstrate the relationship between time and dose on praziquantel concentration in the live brine shrimp.

Materials and Methods

Bioaccumulation assays were performed in 27 separate containers each containing 500 ml of fresh water. Separate containers were prepared identically for each time point (0 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, and 24 hr) at each bath concentration (2.5 g/L, 5 g/L, or 10 g/L). The weight of the praziquantel (Praziquantel USP, Medisca, Inc., Plattsburg, NY 12901) was measured to the nearest 0.001 g. The drug was agitated into solution and maintained with an airstone connected to a standard line for all containers. To each container (except t=0, control for each concentration), 0.25 teaspoon of adult live brine shrimp (Sea Critter, 50 Sea Critters Lane, Key Largo, FL, 33037) was added. Control water containers were maintained throughout the study for each concentration that did not contain shrimp. Total animal counts were performed in triplicate, and were within 10% of each other (4314, 4082, 4185 animals). Average wet weight of each strained sample was 15.98 g (range 15.47 – 16.44

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	2.5 g/L	5 g/L	10 g/L
Time (hrs)	µg/shrimp	µg/shrimp	µg/shrimp
0.25	4.29	8.60	3.37
0.5	5.08	2.73	0.81
1	2.26	3.33	0.92
2	2.08	0.96	0.83
4	3.48	6.82	1.78
8	2.96	2.99	2.44
12	4.47	4.37	3.19
24	7.14	6.52	5.45

Table 1: The average concentration of praziquantel (µg) in each individual brine shrimp measured for each concentration, at 2.5 g/L, 5 g/L, and 10 g/L, and each timepoint.

g). Using these calculations there is an average of 262.38 shrimp/g wet weight. Length was measured in 20 animals and ranged from 5.0 mm – 10.0 mm (mean: 7.325 mm).

Three samples were taken at each timepoint (except t=0): control water (3 ml), treatment water taken from container with shrimp (3ml), and shrimp. Control water was the only sample taken at time 0. All control water samples were taken from the same three containers based on concentration. Shrimp samples were lightly rinsed in freshwater after removal from the treatment water prior to analysis. In each container, survivability of the live brine shrimp was estimated subjectively to the nearest 10%, by one of the co-authors (MK).

High performance liquid chromatography (HPLC) was performed on each of the control water, treatment water, and live brine shrimp samples. Praziquantel was extracted from LBS using a liquid extraction. Previously frozen LBS were thawed and 50 mg placed into screw cap glass tubes. Five hundred microliters of methanol and 10 µl of the internal standard (midazolam 1000 µg/ml) were added to each tube and vortex mixed. Four milliliters of ethyl acetate were added to each tube and then were homogenized using a Power Gen 125 homogenizer (Fisher Scientific, Pittsburgh, PA). Samples were centrifuged at 1000 g and the supernatant was removed to a clean glass tube and evaporated with nitrogen. Residues were re-dissolved in 500 µl of mobile phase and placed into chromatography vials. A 2.5 µl sample was injected into the HPLC.

Results

Praziquantel concentrations in adult *Artemia* were determined based on wet weight. The concentration of praziquantel in shrimp at each dose and time are presented in Table 1. There was a positive time-, but not concentration-dependent difference in praziquantel concentration of adult *Artemia* (Figure 1a-c). A similar pattern of praziquantel uptake was seen in all three treatment groups (Figure 2), in which there was an initial high uptake into shrimp, followed by rapid decline, then a steady increase in concentration for the last 12 hours. The highest concentration of praziquantel in shrimp occurred for 4 hr, 1 hr, and 15 min samples at 2.5 g/L, 5 g/L, or 10 g/L treatments, respectively. All three treatment protocols exhibited a similar trend of increased levels during the terminal 8-12 hrs of the study.

There was no clear concentration- or time-dependent difference in praziquantel concentration in the treatment water (Figure 1a-c). Fluctuations occurred over time in the control water in all bath

concentrations, especially within the first 4 hours of the 5 g/L treatment (Figure 3).

A portion of the sample population survived at each sampling interval: 0 min (100%), 15 min (100%), 30 min (100%), 1 hr (100%), 2 hr (100%), 4 hr (90%), 8 hr (80%), 12 hr (80%), and 24 hr (50%).

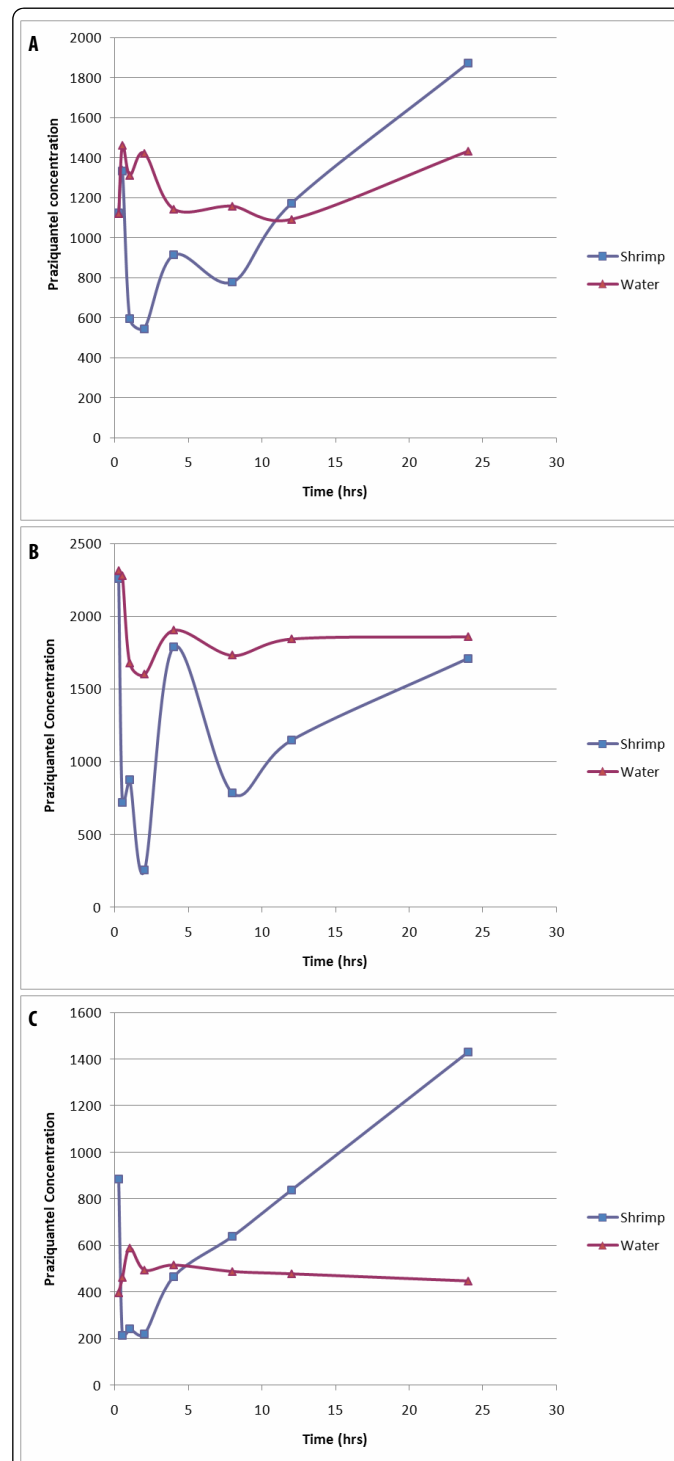


Figure 1: The concentration of praziquantel is measured in adult *Artemia* through bioencapsulation at 2.5 g/L (1a), 5 g/L (1b), and 10 g/L (1c). The concentration of praziquantel in shrimp (squares) is given over enrichment times in µg/g. The concentration of praziquantel in treatment water (triangles) is given over enrichment times in µl/ml.

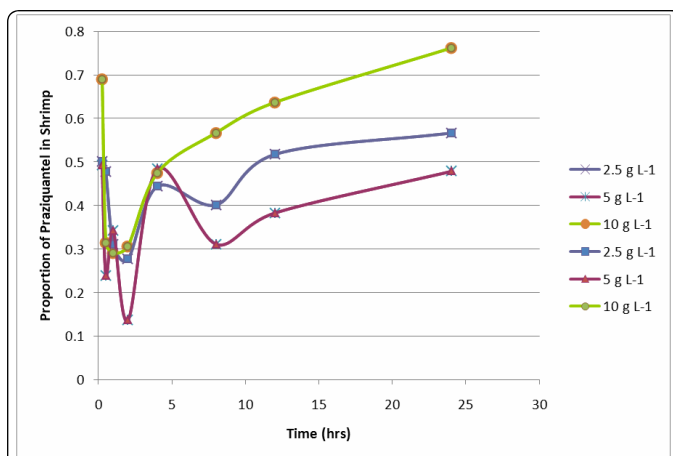


Figure 2: The graph presents the proportion of praziquantel that was available that was taken up by adult *Artemia* through bioencapsulation at 2.5 g/L (square), 5 g/L (triangle), and 10 g/L (circle) enrichment baths.

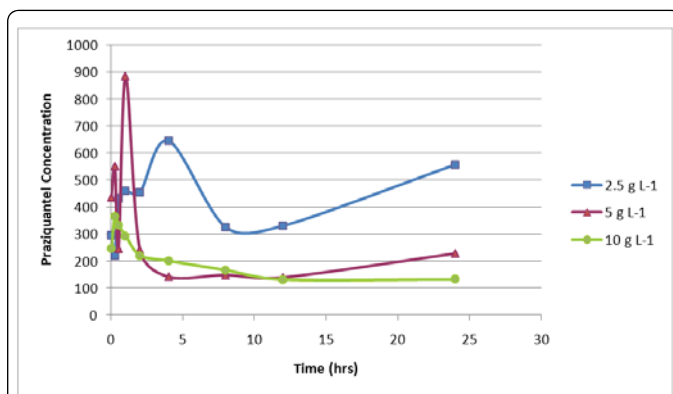


Figure 3: The concentration of praziquantel is measured in control water ($\mu\text{g/ml}$) at 2.5 g/L (square), 5 g/L (triangle), and 10 g/L (circle) treatments.

Discussion

Antimicrobial and anti-parasitic treatments are commonly used for individuals or groups of fish in an aquarium. Ideally, these techniques should be adaptable to the disease of the affected fish, provide therapeutic concentrations, be cost effective, and technically simple to execute. The procedure of bioencapsulation has been widely used in aquaculture to fulfill these criteria. However, specific pharmacological data is lacking for numerous drugs and anecdotal dosing may produce inadequate concentrations, pathogen resistance, or be toxic to the host.

In the present study, adult *Artemia* were exposed to three concentrations of praziquantel in water baths. As expected, the praziquantel concentration in shrimp was higher at the termination of the study when compared to the beginning in all treatments except 5 g/L. There were no concentration-dependent patterns seen in the concentration of praziquantel in the shrimp, in fact the 2.5 g/L treatment had the highest concentration of drug in shrimp at most time points. In each treatment, the concentration of praziquantel in shrimp declined rapidly from the 15 min sample to the 30-minute sample. Also the concentrations fluctuated in each treatment group until the last 12-16 hrs of the study when all groups increased. More interestingly, a pattern is seen when evaluating the total percent of praziquantel that was present in the shrimp over time in which

there is an initial decrease followed by a steady terminal increase in all treatments. The final concentration and percentage of praziquantel in shrimp was higher in the 2.5 g/L than the 5 g/L treatment, but both percentages were lower than the 10 g/L treatment.

This pattern is not consistent with simulations reported for drugs encapsulated in brine shrimp (Aguilar-Aguila et al., 1994). Experiments in *Artemia nauplii* demonstrate increases in several antibiotics over time to a plateau, (Touraki et al., 1996; Gomez-Gil et al., 2001) but no immediate decrease as is seen in the present study. A possible explanation for the observations in the current study include a rapid gut loading of the drug with subsequent active expulsion, followed by either re-uptake by the gut or systemic absorption. Additionally, it is possible that the osmolality of the shrimp in freshwater passively removes the praziquantel after initial exposure. Also there may be unknown age-related or individual feeding rate effects. No studies have been done to determine the effect that stage of development has on the pharmacokinetics in *Artemia*. Regardless, the pattern of initial decline in concentration is repeatable among different concentrations and demonstrates a significant clinical implication; a delay of 15 minutes can cause the concentration to go from therapeutic to non-therapeutic levels depending on target species, animal size, and pathogen.

This study did not achieve an upper plateau, indicating maximum capacity of praziquantel uptake in shrimp was not achieved. Therefore future studies that desire to increase the concentration may be possible. However, the low concentration treatment group attained the highest concentration, therefore maximum drug solubility in water may have been achieved, and any increase of drug concentration in shrimp will likely be attained only through longer bath times not increases in concentration. This may be useful clinically if higher concentrations of praziquantel are desired, such as to accommodate small patient size, inappetence, or MIC of the pathogen in fish. This would also allow a clinician or aquarist to specifically direct therapy to an individual or group of fish by altering time or concentration that the shrimp are exposed to the drug.

There was a time dependent, but not a concentration-dependent increase in mortality of the shrimp, indicating they can tolerate the three concentrations in this study up to 24 hours. The mortality was minimal (less than 20%) during the first 12 hrs indicating that this may be the acceptable maximum length of the encapsulation period and exceeding this limit could reduce the utility of this method. Mortalities may be lessened in the later hours with the enrichment occurring in salt water, as brine shrimp's natural environment is salt water. The effect of salt water on the encapsulation was not assessed and further studies are warranted. The HPLC assays were performed on the shrimp after freezing, and may have been composed of both live and dead shrimp (those shrimp that died during treatment and frozen dead). Therefore, it is also possible that shrimp death alters the drug concentration or effectiveness of targeted delivery, but would not have been detected in this study.

The present study measured praziquantel in the control tanks (without shrimp) and in the treatment tanks (with shrimp). It is important to note that both treatment and control water concentrations varied with time, but similar to the shrimp concentrations, both water concentrations demonstrated a steady terminal increase. The fluctuations were possibly due to a combination of factors including drug precipitating out of solution, human error in dosing, attachment to the air stone, and evaporation.

Praziquantel can be insoluble without agitation; consequently this can restrict its use in a bath making it necessary to do individual dosing, which is often unreasonable in large aquatic collections. This was also a significant concern in the present study; therefore baths were actively monitored and underwent continuous aeration to keep the drug in solution. However, insolubility still resulted which was noted by subjective evaluation and likely contributed to the high variability of the results.

This study provided specific pharmacokinetic data on the bioencapsulation of praziquantel in live brine shrimp that will be useful to fish management personnel. Current reports for delivery of praziquantel to fish in shrimp are not known or commonly performed. However, praziquantel is commonly used as a bath or oral dosing (Carpenter, 2005), but other mechanisms of efficient delivery are desired. This study presents another mechanism to target the treatment of patients. For example, if a goal dose of 10 mg kg⁻¹ in a 15-min enrichment period with praziquantel were chosen, a 100 g fish would need to be given 116 (0.44 g) shrimp treated at 2.5 g/L, 58 (0.22 g) shrimp treated at 5 g/L, and 148 (0.56 g) shrimp treated at 10 g/L. However, waiting just 2 hr, the same fish would need to be given 240 (0.98 g), 520 (1.98 g), and 602 (2.29 g) shrimp, respectively. And waiting 24 hr, the same fish would need to be given 70 (0.27 g), 76 (0.29 g), and 91 (0.35 g) shrimp, respectively. This allows the clinician and management team to use less drug and less shrimp, thereby reducing resources.

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