

Rapid Communication

Range extensions for three new invasive species in Pueblo Reservoir, Colorado, USA: water flea *Daphnia lumholtzi* (G. O. Sars, 1885), calanoid copepod *Eurytemora affinis* (Poppe, 1880), and freshwater bryozoan *Lophopodella carteri* (Hyatt, 1865)

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Received: 3 July 2013 / Accepted: 27 August 2013 / Published online: 10 September 2013

Handling editor: Ian Duggan

Abstract

This is the first report of the occurrence of three invasive species in Colorado, USA: the water flea, *Daphnia lumholtzi* (G. O. Sars, 1885); calanoid copepod, *Eurytemora affinis* (Poppe, 1880); and a freshwater bryozoan, *Lophopodella carteri* (Hyatt, 1865). Analysis of monthly plankton samples taken in the Pueblo Reservoir from 2008–2010 shows recurring presence of *D. lumholtzi* and *E. affinis*, indicating range extension and establishment of the two zooplankters; *L. carteri* was collected in abundance in 2008 suggesting its success in the reservoir. These findings could be invaluable for determining long-term implications and management decisions pertaining to the three invasive species.

Key words: exotic species; zooplankton; bryozoan; seasonal densities

Introduction

Organisms may successfully invade a new environment if able to occupy a vacant niche (Dzialowski et al. 2000), by out-competing a native species (Dobberfuhl and Elser 2002), or by experiencing reduced predation vulnerability due to a morphological or behavioral advantage (Work and Gophen 1999). Invasions of non-native aquatic species can pose a significant threat to the existing community and subsequently affect the food chain (Dzialowski et al. 2000). It is important to monitor non-indigenous species in order to make management decisions and to predict long-term implications of their presence.

Daphnia lumholtzi (G. O. Sars, 1885) is a cladoceran zooplankter that can be easily distinguished from the native daphnids by elongated helmet and tail spines, pointed fornicies, and a spined ventral carapace margin (Havel and Hebert 1993) (Figure 1A). Indigenous to Africa,

Asia, and Australia, *D. lumholtzi* was most likely introduced to the United States when *Lates niloticus* (Linnaeus, 1758), the Nile perch, was brought from Lake Victoria, Africa, to stock Lake Fairfield in Texas in 1983 (Havel and Hebert 1993). Since being first reported in 1991 by Sorensen and Sterner (1992), it has successfully invaded much of south-central U.S., the Great Lakes, and also parts of the West Coast (Wittmann et al. 2013). Studies show high correlation between elevated water temperatures in late summer and the presence of *D. lumholtzi* (Dzialowski et al. 2000; Havens et al. 2011).

The calanoid copepod, *Eurytemora affinis* (Poppe, 1880), is a dominant component of the planktonic and epibenthic communities in salt-marsh, brackish, and estuarine waters. It passively disperses via river systems and has invaded many North American, European, and Asian freshwater habitats (Lee 1999). *Eurytemora affinis* is identified as having antennules with only 24 segments,

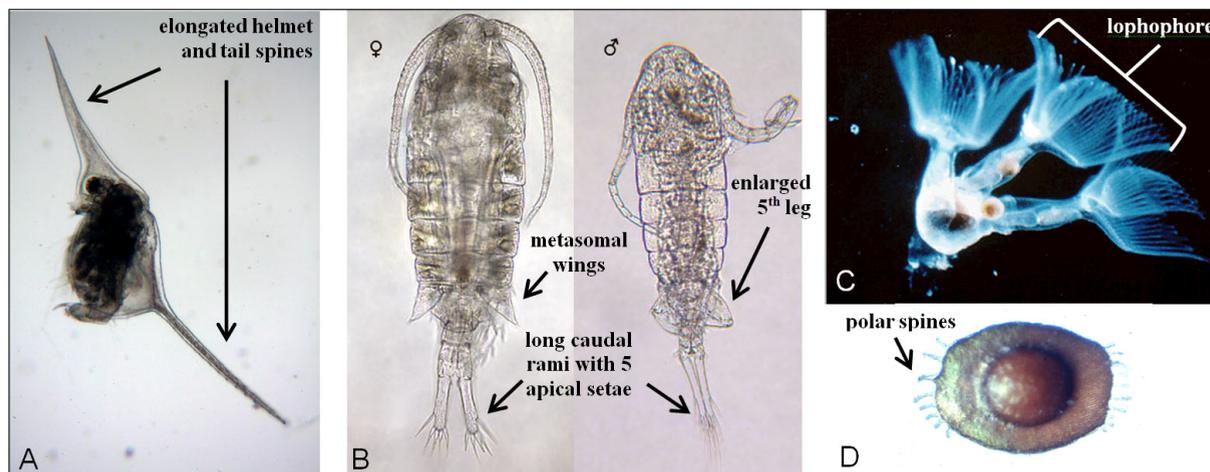


Figure 1. Images of the three invasive species: **A.** *Daphnia lumholtzi*; **B.** *Eurytemora affinis*; **C.** *Lophopodella carteri*; **D.** *L. carteri* statoblast. Image A courtesy of Beaver et al. (2010); Image B courtesy of Lesko et al. (2003); and images C and D courtesy of Timothy Wood (2013).

maxillipeds are subequal to the body width in lateral view, female genital segment with pointed processes, long caudal rami that are more than 3 times the width, with five apical setae, female caudal rami covered in hair and spinules, fifth legs that lack endopods, pointed metasomal wings in females, and fifth legs that are greatly enlarged in males (Wilson 1959; Lesko et al. 2003; Reid and Williamson 2010) (Figure 1B).

The native range of the freshwater bryozoan, *Lophopodella carteri* (Hyatt, 1865), includes Europe, Asia, and Africa (Wood and Marsh 1996). It was introduced to North America when aquatic plants were imported in the 1930s (Wood and Marsh 1996). This invasive macrofouler is often locally abundant and experiences prolific growth in shallow water with solid substrata (Lauer et al. 1999). *Lophopodella carteri* has distinctive statoblasts with a broad oval shape and polar hooked spines (Wood et al. 2006) (Figures 1C and D).

Pueblo Reservoir is located in the Arkansas River Basin situated in the southeastern region of Colorado (Figure 2). It is a 441,189,323 m³ (357,678 acre-ft.) multipurpose facility that was completed as part of the U. S. Bureau of Reclamation effort called the “Fryingpan-Arkansas Project” in 1975 (Ortiz et al. 1998). When full, it stretches approximately 14.5 km in length with widths ranging from 0.5–3.5 km and a maximum depth of over 47 m at the dam (Mast and Krabbenhoft 2010). Storage usually peaks each

year in April, decreasing in summer and early autumn due to high irrigational demands and decreased inflow (Mast and Krabbenhoft 2010). The reservoir is stocked regularly and supports fisheries of both warm- and cold-water species (Mast and Krabbenhoft 2010). Our efforts were part of a surveillance program established to detect zebra mussels, *Dreissena polymorpha* (Pallas, 1771), and quagga mussels, *Dreissena bugensis* (Andrusov, 1897).

Materials and methods

To sample planktonic organisms, vertical hauls (15 m) were taken monthly from 2008–2010 using a Wildco[®] plankton net (500 mm mouth, 2 m length, 63 μm mesh) in Pueblo Reservoir (Figure 2). Multiple sites were sampled during warmer months. Sites and dates sampled are shown in Table 1. Samples were preserved in 70% ethanol and analyzed as described in Beaver et al. (2010).

To collect epibenthic organisms, three removable substrata were fixed at two sites in late May 2008 (Figure 2). Each consisted of nylon ropes (8 mm diameter) reaching to bottom depths with perforated plastic pipes (25 cm length, 9.5 cm diameter) and/or folded plastic coated screens (22 cm × 22 cm, 1 cm² openings) attached every 1–4 m. Substrates were removed in October 2008 and percent coverage of organisms was estimated.

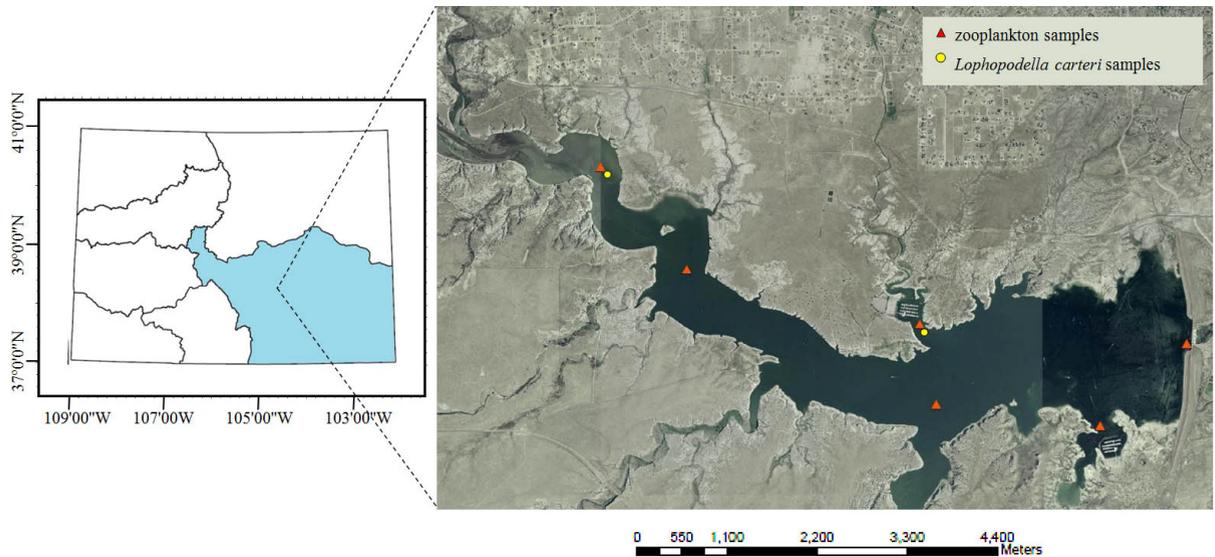


Figure 2. Site map of Pueblo Reservoir, Colorado in the Arkansas River basin (shown in blue). Six sites were sampled for zooplankton from 2008–2010. *Lophopodella carteri* was collected from two sites in 2008. For more extensive distribution maps for our three invasive species, we recommend visiting <http://nas.er.usgs.gov/>.

Samples were preserved in 70% ethanol. Species verification of *L. carteri* from statoblasts was completed by Timothy Wood, Wright State University, Dayton, OH.

Results

No dreissenid mussels were detected in either the planktonic samples or the epibenthic samples; however, Pueblo Reservoir is the first recorded site in Colorado for three invasive species: the water flea, *Daphnia lumholtzi* (G. O. Sars, 1885); calanoid copepod, *Eurytemora affinis* (Poppe, 1880); and freshwater bryozoan, *Lophopodella carteri* (Hyatt, 1865). The two invasive zooplankters show recurring high densities during the summer and fall months in the reservoir. *Daphnia lumholtzi* has highest densities of approximately 2.5–5.0 L⁻¹ from July–September and *E. affinis* is at peak density of about 0.25–0.75 L⁻¹ twice a year with the first peak in May or June and the second in later September or November (Figure 3). *Daphnia lumholtzi* displays peaks of high densities that are offset temporally from its native cladoceran counterparts, whose highest densities occur in earlier spring and summer (Figure 3). Similarly, native calanoid copepod species show highest densities in early

spring and summer. This is a temporal offset from *E. affinis* at its peaks; however, overlaps between the native species and *E. affinis* do occur throughout the season (Figure 3).

Percent coverage of *L. carteri* was estimated to be approximately 80% on the substrates. *Lophopodella carteri* formed colonies on all parts of the substrata including the pipes, screens, and most of the ropes.

Discussion

Both *D. lumholtzi* and *E. affinis* are considered established as they show recurring presence after overwintering periods in each of the sampling years (Figure 3). Their establishment comes with many concerns. *Daphnia lumholtzi* has morphological adaptations to deter predation, namely the elongated helmet and tail spines. These morphological changes can be induced under a variety of circumstances such as predator kairomones (Kolar and Wahl 1998; Dzialowski et al. 2003; Engel and Tollrian 2009), temperature (Sorensen and Sterner 1992; Yurista 2000), and insecticides (Hanazato 1999). Lemke et al. (2003) found *D. lumholtzi* was consumed by juvenile bluegill, white and black crappie, and white bass; however, gizzard shad, emerald shiner, and freshwater

Table 1. Zooplankton sample dates and sites in Pueblo Reservoir. GPS coordinates are as follows for each site: PR-1 (13 523965E, 4235561N); PR-2 (13 520861E, 4234811N); PR-3 (13 517771E, 4236498N); PR-4 (13 516705E, 423776N); PR-5 (13 520658E, 4235802N); PR-6 (13 522893E, 4234541N).

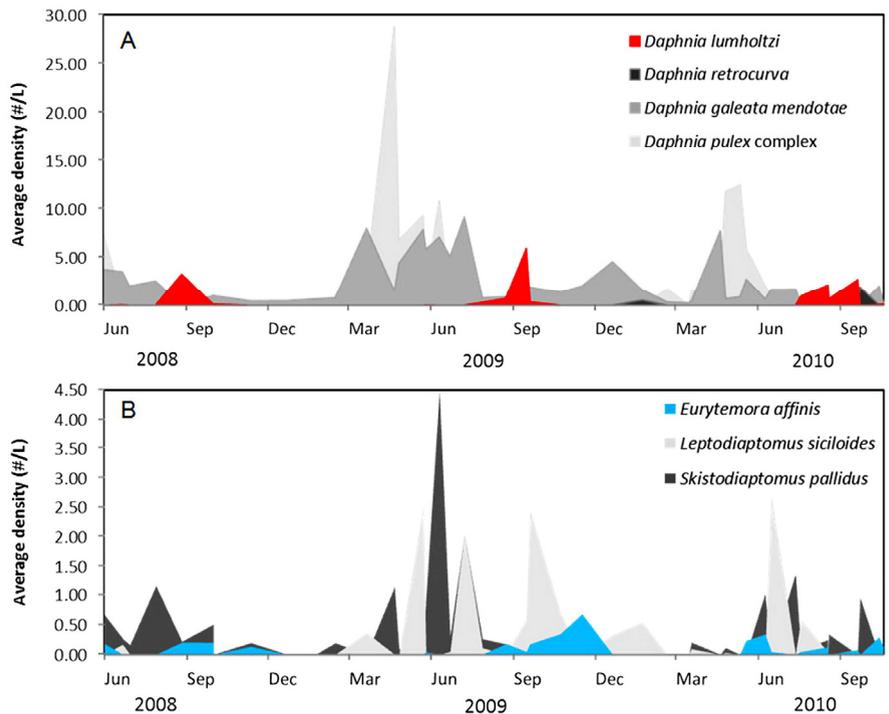
Sample Date	Sites Sampled					
	PR-1	PR-2	PR-3	PR-4	PR-5	PR-6
9-Jun-2008	X	X	X	X	X	X
30-Jun-2008	X	X		X	X	X
8-Jul-2008	X	X	X	X	X	X
6-Aug-2008	X	X	X	X	X	X
4-Sep-2008	X	X	X	X	X	X
9-Oct-2008	X	X	X	X	X	X
20-Nov-2008	X	X	X	X	X	X
30-Dec-2008					X	
31-Jan-2009					X	
22-Feb-2009					X	
29-Mar-2009					X	
29-Apr-2009					X	
4-May-2009	X	X	X	X	X	X
31-May-2009					X	
3-Jun-2009	X	X	X	X	X	X
18-Jun-2009					X	
30-Jun-2009	X	X	X	X	X	X
16-Jul-2009						X
5-Aug-2009	X	X	X	X	X	X
31-Aug-2009					X	
23-Sep-2009	X	X	X	X	X	X
27-Sep-2009					X	
31-Oct-2009					X	
24-Nov-2009					X	
28-Dec-2009					X	
30-Jan-2010					X	
27-Feb-2010					X	
23-Mar-2010					X	
26-Mar-2010	X	X	X	X	X	X
27-Apr-2010					X	
3-May-2010	X	X	X	X	X	X
19-May-2010					X	
26-May-2010	X	X	X	X	X	X
16-Jun-2010					X	
23-Jun-2010	X	X	X	X	X	X
20-Jul-2010					X	
26-Jul-2010	X	X	X	X	X	X
25-Aug-2010	X	X	X	X	X	X
26-Aug-2010					X	
27-Sep-2010	X	X	X	X	X	X
30-Sep-2010					X	
21-Oct-2010	X		X	X	X	X
26-Oct-2010		X				

drum species showed significant negative selectivity. Of those species, black crappie, *Pomoxis nigromaculatus* (Lesueur in Cuvier and Valenciennes, 1829), and bluegill, *Lepomis macrochirus* (Rafinesque, 1819), only make up 1% each of the relative fish abundance in Pueblo Reservoir and gizzard shad, *Dorosoma cepedianum* (Lesueur, 1818), contributes 11% (McGree 2012). The fish with the greatest relative abundance in Pueblo Reservoir is the walleye, *Sander vitreus* (Mitchill,

1818), though no work to date has been published on whether larval or juveniles of this species consume *D. lumholtzi*. While some fish species are known to capture *D. lumholtzi*, their selection for it is altered by the presence of other daphnid choices.

Kolar and Wahl (1998) determined that juvenile bluegill prefer *Daphnia pulex* (Leydig, 1860) over *D. lumholtzi* when given the choice. *Daphnia pulex* (complex) is the dominant cladoceran

Figure 3. Monthly variation in the average densities of **A)** resident daphnids and invasive *Daphnia lumholtzi* and **B)** resident calanoid copepods and invasive *Eurytemora affinis*. Averages were calculated when multiple sites were sampled in the same day; n = 6 unless otherwise noted in Table 1.



species in Pueblo Reservoir (Figure 3). Laboratory experiments examining competition between *D. lumholtzi* and *D. pulex* grown together shows a reduction in biomass for both species (*D. lumholtzi* was reduced to 55% and *D. pulex* was reduced to 17% of control treatment biomass) with combined production significantly lower than the native daphnid production on its own (Dobberfuhl and Elser 2002). This reduction in overall biomass was observed in Alamo Lake, AZ, where *D. lumholtzi* was the dominant daphnid. Zooplankton was lower by two orders of magnitude and algae were higher by one order of magnitude than the other, non-invaded reservoirs in the study (Dobberfuhl and Elser 2002). From 1991–2009, Martinez et al. (2010) surveyed 27 Colorado reservoirs and lakes (not including Pueblo Reservoir) and *D. lumholtzi* was not found in any samples.

Dzialowski et al. (2000) proposed that *D. lumholtzi* is likely transported when their diapausing egg stages, ephippia, are attached to boats, ropes, or macrophytes. The lack of adult *D. lumholtzi* in the other Colorado reservoirs and lakes may be because the species has yet to be introduced or,

upon introduction, environmental conditions were unsuitable for their establishment. The absence of adults is inconclusive, however, and if dispersal of ephippia has occurred, potential populations may exist in the sediments and develop if conditions become more favorable (Dzialowski et al. 2000).

Eurytemora affinis is a major concern because it is known to be a carrier of harmful pathogens such as *Vibrio cholerae* in its native ranges (Huq et al. 1983; Lee et al. 2007; Winkler et al. 2008). An additional concern is its ability when conditions are stressful to produce resting eggs that are resistant to desiccation, giving the copepod the ability to persist in an invaded environment (Lee and Bell 1999). Much work has been done on the organism's physiology. Specifically, its tolerance of broad ranges of salinity and the selection mechanisms that yield populations of freshwater-tolerant *E. affinis* have been the topics of many publications (Lee 1999; Lee and Bell 1999; Lee et al. 2007) making it an especially interesting animal with regard to evolutionary research.

Little is known of *E. affinis*' ecological interactions with predators and prey in the

invaded freshwater systems but Gulati and Doornekamp (1991) provided a study of a lake system in the Netherlands, Rhine River Delta Region, which was created in 1987 when an enclosure was made that cut off input from estuarine tidal influence. In about a year, the lake changed from high chloride concentrations ($>13,000 \text{ mg L}^{-1}$) to a more freshwater regime with chlorides of $300\text{--}600 \text{ mg L}^{-1}$ (Gulati and Doornekamp 1991). Flora and fauna more typical of freshwater nearly completely replaced the former estuarine counterparts; however, *E. affinis* was found both before and after the lake was enclosed (Gulati and Doornekamp 1991). Its peak densities occurred when cladocerans were scarce and declines in *E. affinis* abundance coincided with increased *D. pulex* and *D. galeata* (Gulati and Doornekamp 1991), two cladocerans that are also found in Pueblo Reservoir (Figure 3). Fish predation on *E. affinis* by species of stickleback, perch, and pikeperch was thought to also contribute to the population's seasonal decline (Gulati and Doornekamp 1991). *Eurytemora affinis* consumed both detritus and algae, and was likely the main controlling factor in primary production of phytoplankton and a large share of seston mass (Gulati and Doornekamp 1991).

Lophopodella carteri was collected in Pueblo Reservoir and appears to be thriving. Its presence comes with potential concerns because it exhibits macrofouling tendencies with prolific growth and localized abundance (Lauer et al. 1999). Its substrate preferences, feeding mechanisms, expansive settlement, and planktonic larvae mirror those traits of the zebra mussels, *D. polymorpha*, which may raise some alarm (Lauer et al. 1999). On the other hand, it may be a potential ally against the establishment of the zebra mussel as Lauer et al. (1999) demonstrated that *L. carteri* may play a role in inhibiting the mussels by: 1) producing microcurrents when feeding that can physically displace the larvae during their settling attempts, 2) densely covering substrata thereby deterring settlement, and 3) killing or repelling settling zebra mussels with larvotoxic allelochemicals in their coelomic fluid. No dreissenid mussels were detected on our removable substrata where *L. carteri* was found. Not only does *L. carteri* exhibit localized abundance but it also has unique statoblasts which immediately sink and can remain dormant for extended periods of time when conditions are unfavorable (Wood and Marsh 1996). When the optimal environment returns, they can begin new bryozoan colonies (Wood and Marsh 1996). These statoblasts are resistant to desiccation, germinating

more readily if dried out, providing the species with the ability to resist drought conditions (Wood and Marsh 1996). At this point, it is unclear how the bryozoan came to arrive in Pueblo Reservoir, but studies in other parts of the world suggested that the introduction of *L. carteri* was due to statoblast dispersal (Sanzhak et al. 2011). *Lophopodella carteri* does appear to co-occur with native species such as *Plumatella reticulata* (Wood, 1988) and *Plumatella fungosa* (Pallas, 1768) but little is known of their interactions in Pueblo Reservoir.

Many attributes make Pueblo Reservoir an ideal environment for the three new invasive species. Pueblo Reservoir experiences heavy boating traffic (approximately $40,000 \text{ yr}^{-1}$ launched from the boat ramps - B. Henley, personal communication, June 13, 2013), providing dispersal vectors for invasive organisms. In the Arkansas River Basin where Pueblo Reservoir is located, streamflow regimes can be broken into three categories: runoff from snowmelt (May-June), post runoff (July-September), and low flow (October-April) (Ortiz et al. 1998). Because the reservoir is located at a plains elevation but receives cold water influx from snow runoff, multiple temperature niches are available throughout the year. High abundance of *D. lumholtzi* is consistently associated with warmer temperatures and larger surface area with high boat traffic in the literature (Havel et al. 2005; Dzialowski et al. 2000; Havel et al. 1994; Havel et al. 2002). Reservoirs, especially those located in arid climates, may also have salinities that are slightly elevated. This may provide adaptation havens for organisms such as *E. affinis* where natural selection can result in populations with different osmotic tolerances (Lee 2002; Havel et al. 2005). *Lophopodella carteri* tends to be found in shallow alkaline waters on a variety of solid substrates ranging from rocks and pier posts to macrophytes and mussel shells (Lauer et al. 1999). While its colonies can sometimes be described as a "continuous gelatinous layer", it experiences this prolific growth in temperatures between $15\text{--}28^\circ\text{C}$ (Lauer et al. 1999). Pueblo Reservoir appears to have the conditions necessary for the establishment of these new invasive species but additional research is needed. In summary, all three invasive species are intriguing components in the Pueblo Reservoir ecosystem and our baseline data should help provide direction for continuing research to better understand how their presence will affect the existing communities.

Acknowledgements

We thank the following for their contributions in funding and support: James Melby, Colorado Parks and Wildlife, Pueblo, CO; Timothy Wood, Wright State University, Dayton, OH; Board of Pueblo County Commissioners, Pueblo, CO; Board of Water Works of Pueblo, Pueblo, CO; Lower Arkansas Valley Water Conservancy District, Rocky Ford, CO; U.S. Bureau of Reclamation, Denver, CO. We also greatly appreciate the editorial comments and suggestions provided by the reviewers.

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