

Selectivity of *Incilius valliceps* (Anura: Bufonidae) tadpoles on freshwater zooplanktonSelectividad de los renacuajos de *Incilius valliceps* (Anura: Bufonidae) sobre el zooplancton de agua dulce

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ABSTRACT

Background. The dietary preferences of tropical tadpoles and the way they interact with zooplankton often go unstudied in aquatic ecosystems. **Goals.** We investigated the interactions between *Incilius valliceps* tadpoles and a freshwater zooplankton community found in an artificial aquatic pond located on Cozumel Island in Quintana Roo, Mexico. **Methods.** We determined zooplankton abundance, richness, the Shannon-Wiener diversity index, and the proportion of each zooplankton species in the environment. We also evaluated characteristics of the tadpole population such as body size, abundance, and proportion of prey ingested as determined by stomach content analysis. We used the Jacobs selectivity index to determine tadpole prey of preference. **Results.** The rotifers *Anuraeopsis fissa* and an unidentified species of Bdelloidea group were dominant among the eight zooplankton species consumed. The selectivity index showed that *I. valliceps* tadpoles preferred to consume macro-zooplankton (ostracods and cladocerans) instead of the rotifer species available. Tadpoles positively chose *Cypridopsis vidua* throughout their growth and development and *Moina wierzejskii* during their early developmental stages, and negatively selected the rotifer species of the one unidentified species of Bdelloidea, *A. fissa*, and *Epiphanes brachionus*. In the presence of tadpoles, *Keratella americana*, *Lepadella patella*, and *Lecane luna* were either present in extremely low abundances or not found in the aquatic community, and *M. wierzejskii* produced males and resistant eggs. **Conclusions.** The findings of this study clarify the dietary needs of tropical tadpoles and their role in aquatic ecosystems.

Key words: Aquatic, feeding, freshwater, preferences, tropical zooplankton.

RESUMEN

Antecedentes. Las preferencias de alimento de renacuajos tropicales y la manera en que interactúan con el zooplancton, han sido poco estudiadas en los ecosistemas acuáticos. **Objetivos.** Se estudiaron las interacciones entre renacuajos de *Incilius valliceps* y una comunidad de zooplancton de agua dulce en un sistema acuático artificial de Cozumel, Quintana Roo, México. **Métodos.** Se determinó la abundancia y riqueza de zooplancton, la diversidad de Shannon-Wiener y la proporción de cada especie de zooplancton en el ambiente. La preferencia de presas de los renacuajos se determinó con el índice de selectividad de Jacobs. **Resultados.** Los rotíferos *Anuraeopsis fissa* y una especie no identificada de bdeloideo, fueron dominantes entre las ocho especies de zooplancton presentes en el ambiente acuático. El índice de selectividad mostró que los renacuajos consumieron con mayor frecuencia al macrozooplancton (ostrácodos y cladóceros) en lugar de las especies de rotíferos disponibles: seleccionaron positivamente a *Cypridopsis vidua* durante todo su crecimiento y desarrollo y a *Moina wierzejskii* en las etapas más tempranas de su desarrollo. Se registró selectividad negativa para la especie no identificada de bdeloideo, *A. fissa* y *Epiphanes brachionus*. En la presencia de los renacuajos, *Keratella americana*, *Lepadella patella* y *Lecane luna* se ausentaron de la comunidad, o fueron poco abundantes y *M. wierzejskii* produjo machos y huevos de resistencia. **Conclusiones.** Los resultados de este trabajo aportan información en cuanto la dieta de renacuajos tropicales y su papel en los ecosistemas acuáticos.

Palabras claves: Acuático, alimentación, agua dulce, preferencias, zooplancton tropical.

INTRODUCTION

The toad *Incilius valliceps* (Wiegmann, 1833) is common on Cozumel Island, and their distribution ranges widely throughout the states of Oaxaca, Chiapas, and the Yucatan Peninsula in Mexico (Ochoa-Ochoa *et al.*, 2006). Although this toad is not yet considered threatened or endangered (Rojas-Canales & Ríos-Valdez, 2012), the decline of amphibian species, in general, is of great concern, especially in Latin America (Young *et al.*, 2000). Information regarding tadpole food preferences is scarce, but they often act as detritivores, herbivores, carnivores, or omnivores (Altig *et al.*, 2007). By examining the gut contents, we can better understand the dietary needs of tadpoles and thus obtain a complete understanding of the ecological role that they play. The ecological behavior of freshwater zooplankton is affected by a wide variety of biotic and abiotic factors. Predation is known to influence the behavior of zooplankton and produces effects that can be noticed in the population size, the production of ephippial (resting) eggs, and the distribution of zooplankton throughout the limnetic zone (Lampert & Sommer, 2007). The effect of fish, copepod, freshwater shrimp, and insect larva predation on communities of zooplankton has been well documented (O'Brien, 1979; Vijverberg *et al.*, 1990; DeVries & Stein, 1992; Gliwicz & Umaña, 1994; Jawahar-Ali *et al.*, 1996; Fernando, 2002; Romare *et al.*, 2003; Cervantes-Martínez, 2005). However, zooplankton interaction with tadpoles remains largely unstudied; only a few studies have investigated this relationship, such as the one performed by Hamilton *et al.* (2012) that identified the predator-prey relationship that exists between cladocerans (*Daphnia pulex* Leydig, 1860) and tadpoles (*Rana aurora* Baird & Girard, 1852) and recognized the importance of cladocerans in the tadpole diet. Given the lack of information related to the feeding habits of tropical tadpoles and their relationship with zooplankton, we aimed to identify the dietary preferences of *I. valliceps* tadpoles and the way in which their presence influenced zooplankton richness and abundance in a small artificial pond, where these organisms are present throughout the year.

MATERIALS AND METHODS

Sample collection. We undertook biological collections from one artificial pond located on the campus of the University of Quintana Roo (20° 29' 18.3" N and 86° 56' 22.8" W) on Cozumel Island, Mexico, between June 2011 and January 2012, covering the entire rainy season which favors anuran oviposition (Cedeño-Vázquez *et al.*, 2006). The volume of the pond remained nearly constant (4 m³), had a depth < 1.5 m, and was filled exclusively with rain water.

We collected zooplankton samples monthly using a van Dorn bottle; we filtered 5 L of water through a zooplankton net with a mesh size of 45 µm. We took duplicate samples at a depth of 0.5 m and preserved them according to standard methods for further observation (Eliás-Gutiérrez *et al.*, 2008).

We took samples of *I. valliceps* tadpoles once a month for three months, using a mosquito-netting-like sieve to collect as many tadpoles as possible within 5 minutes. We introduced the net to the center of the system and, using a weight, made it rest on the bottom. After the specified time had passed, we removed the net in a swift upward motion trapping any tadpoles that had rested on the bottom and those found in the water column. We captured between 15 to 30 individuals in the months in which tadpoles were present (July, September, and

October), with the exception of July (only six individuals). We preserved all tadpoles following standard methods for the posterior gut content analysis (Dutra & Callisto, 2005).

Laboratory. To evaluate the prey availability in the system, we analyzed the monthly average zooplankton abundance and diversity (S). We estimated the abundance of each zooplankton species using total counts (Lewis, 1979), or the aliquot method for those samples that had extremely large quantities of zooplankton (Oscoz *et al.*, 2006). In order to identify zooplankton species, we used taxonomic keys from Koste (1978), Victor (2002), Kutikova (2002), and Eliás-Gutiérrez *et al.* (2008). We calculated the availability of each zooplankton species as the proportion of each species, relative to the total number of individuals pertaining to the zooplankton community. We quantified the diversity of zooplankton species using the Shannon-Wiener Index (Shannon & Weaver, 1949). Shannon-Wiener Index: $(H') = -\sum p_i \ln p_i$, where p_i is the relative abundance of the species i .

In order to describe tadpole predation on zooplankton, we recorded morphological magnitudes for each tadpole: body length, tail length, and total length (Altig & McDiarmid, 1999). The intestinal contents of the tadpoles were analyzed according to Dutra & Callisto (2005), and the proportional abundance of zooplankton species in the tadpole intestines was estimated by total count.

We calculated the selectivity of zooplankton species by each tadpole as a monthly average using the Jacobs selectivity index (Jacobs, 1974): $D = (r-p) / ((r+p)-2p)$, where r is the proportion of a specific prey species in the gut contents and p is the proportion of the specific prey species in the environment. This index (D) ranges from -1 (negative selection), 1 (positive selection), and 0 (no selection) (Lampert & Sommer, 2007).

We performed a simple linear regression in Statgraphics Centurion v. 10.0 to test if tadpole size played a role in the amount of zooplankton consumed. We transformed the data using $\log(x+1)$.

RESULTS

Eight zooplankton species were identified as available in the medium: six rotifers: *Epiphanes brachionus* (Ehrenberg, 1837), *Anuraeopsis fissa* Gosse, 1851, *Keratella americana* Carlin, 1943, *Lepadella patella* (Müller, 1773), *Lecane luna* (Müller, 1776), and an unidentified species of Bdelloidea group, one cladoceran, i.e., *Moina wierzejskii* (Richard, 1895), and one ostracod, i.e., *Cypridopsis vidua* (Müller, 1776).

In the zooplankton community, the Shannon-Wiener diversity index (H') ranged from 0.015 to 1.27 (Fig. 1). In August, October, and December, we confirmed the presence of dominant species (the species of unidentified bdelloideo and *A. fissa* respectively) and lower values in H' . Meanwhile, the months with higher richness (S) and diversity were June (S= 5, H' =1.27), September (S=5, H' =0.98), and November (S=5, H' = 1) (Fig. 1).

The availability of zooplankton species in the artificial aquatic system varied throughout the study period. *E. brachionus* was greatly available in the month of July (68.71%), while *A. fissa* was available in all months, but especially in October (99.81%), December (91.52%), and January (83.25%). *K. americana*, *L. luna*, and *L. patella* were the species with the least availability throughout the period of study (maximum: 0.06%, 0.01%, and 34.78%, respectively). *C. vidua* was also

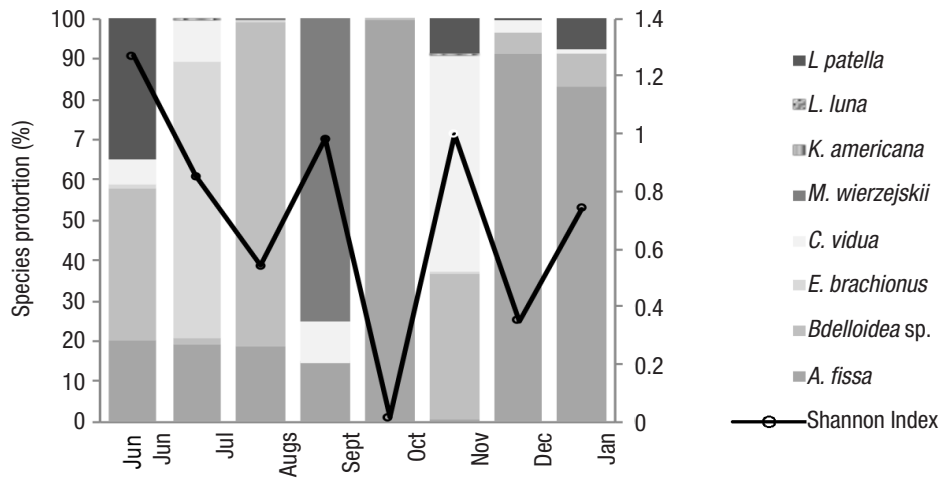


Figure 1. Proportion of every zooplankton species in the environment (for every month (columns) of the study and the corresponding Shannon-Wiener Index values (rows), found in an artificial aquatic pond on Cozumel Island, Mexico.

available throughout the study period in small proportions with the highest availability recorded in November (53.43%). *M. wierzejskii* was only available in August (0.55%) and September (74.95%) (Fig. 1).

Only *Bdelloidea sp.*, *C. vidua*, *M. wierzejskii*, *A. fissa*, and *E. brachionus* were consumed by *I. valliceps*, and the species found in higher proportions in the tadpole gut contents were *C. vidua* (July 83.19%, September 26.54%, and October 36.28%) and *M. wierzejskii* (September 69.63% and October 13.5%) (Fig. 2).

In July, *C. vidua* was more abundant in the tadpole guts (83.19%) when the availability was relatively low in the environment (10.64%). While in October, *C. vidua* was found frequently in the gut contents of the tadpoles (36.28%), but occurred rather rarely in the pond (0.02%). The tadpoles examined in September mainly contained *M. wierzejs-*

kii (69.63%), which was also the most abundant zooplankton species in the environment (74.95%). The species of *bdelloidea* was not very abundant in the environment when tadpoles were present (July: 1.26%, September: 0.38%, and October: 0.08%) and was also only observed in the gut contents in small proportions (July: 0.14%, September: 3.26%, and October: 12.19%) *A. fissa* was abundant in all three months (July: 19.26%, September: 14.34%, and October: 99.88%), but was absent in all the gut contents with the exception of October (2.75%) (Figs. 1-2).

The Jacobs selectivity index provided insight into the feeding preferences of *I. valliceps* tadpoles in July, September, and October. *C. vidua* was positively selected; while *M. wierzejskii* changed from not being actively selected (no selection) in September to being positively selected in October; *E. brachionus*, *bdelloidea*, and *A. fissa* were altogether negatively selected (Fig. 3).

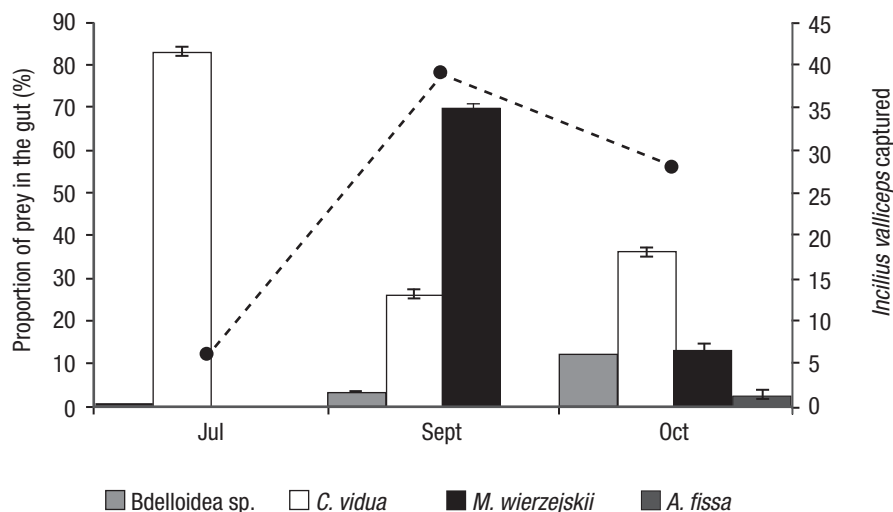


Figure 2. Proportion of each zooplankton prey species present in the tadpole gut contents in each month (columns) and the number of *Incilius valliceps* tadpoles captured in each month (rows), found in an artificial aquatic pond on Cozumel Island, Mexico.

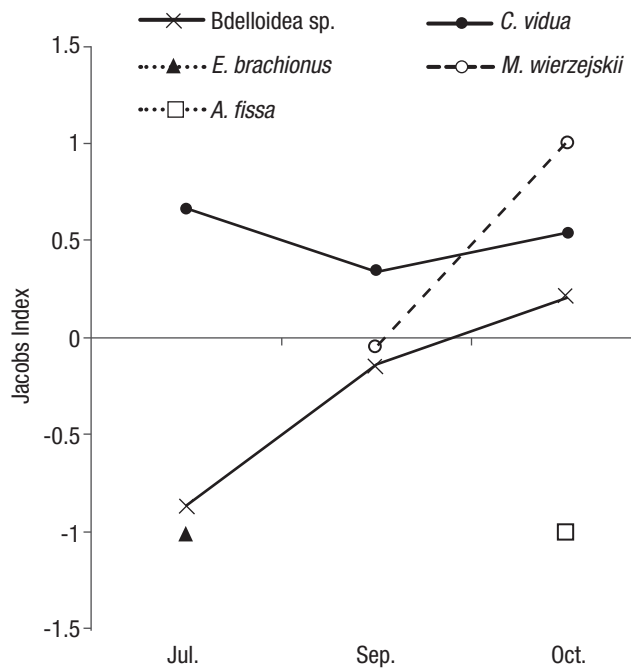


Figure 3. Jacobs selectivity index (monthly average) for every zooplankton species preyed on by the tadpoles each month, found in an artificial aquatic pond on Cozumel Island, Mexico.

The simple linear regression applied revealed the existence of a statistically significant relationship between tadpole size and the number of *C. vidua* ($R^2 = 0.215$, $p = 0.00256$; Fig. 4a), *M. wierzejskii* ($R^2 = 0.1604$, $p = 0.02021$; Fig. 4c), and bdelloidea consumed ($R^2 = 0.2609$, $p = 0.0175$; Fig. 4b). Larger tadpoles ingested more *C. vidua* and bdelloidea and smaller tadpoles consumed slightly more *M. wierzejskii*.

DISCUSSION

The abundance and presence of tadpoles in aquatic systems is determined by where adult anurans choose to lay their eggs. Tadpoles are entirely aquatic organisms, meaning that adults must lay their eggs in an aquatic medium where water and resources are abundant, to ensure their development and metamorphosis (Cedeño-Vázquez *et al.*, 2006).

The artificial aquatic system examined in this study contains an established zooplankton community, which explains the relative stability in the number and type of species present. Aquatic systems often reach equilibrium in terms of the number of species present as they age (Dodson *et al.*, 2007; Alfonso *et al.*, 2010). This particular aquatic system is approximately eight years old and, as documented in this study, *A. fissa*, bdelloidea, and *C. vidua* are always present (established species). Species richness each month was always between four to six species. This means that a constant and predictable supply of food is available for the tadpoles inhabiting this pond.

The Jacobs selectivity index applied to the gut contents of the tadpoles showed that the tadpoles of *I. valliceps* clearly preferred *C. vidua*

throughout the duration of this study. The positive values of the linear regression between the size of the predator and the amount of *C. vidua* ingested suggest that the tadpoles preferred to consume this ostracod throughout their entire growth and development. The importance of *C. vidua* to the tadpole diet is a reason to perform experimental tests in a controlled environment.

Furthermore, the relationship between tadpole length and the number of *M. wierzejskii* consumed demonstrates that this species was highly consumed by the young tadpoles, but consumption of cladocerans diminished as the tadpoles matured and the availability changed. Sousa Filho *et al.* (2007) have previously reported slight differences in diet, depending on the size and larval stage of tadpoles.

Although ostracods are known for partially swimming and crawling along the bottom of aquatic systems (Cohen *et al.*, 2007), *C. vidua* is an efficient swimmer that can enter into the limnetic zone, and is relatively large when compared to rotifer species (Victor, 2002), which may cause this species to be easily seen by the tadpoles. As has been recorded in fish (visual predators), larger prey are positively selected in an effort to conserve energy (Lynch, 1979; Owili & Omondi, 2003; Gallardo Alanis *et al.*, 2009). This may explain why tadpoles positively selected the larger zooplankton species (*C. vidua* and *M. wierzejskii*) in this artificial aquatic system.

Even though the tadpoles did not exhibit a clear preference for bdelloidea throughout the study, individuals measuring 4.7 cm consumed a greater quantity of this rotifer species than their counterparts did. Bdelloidea, *A. fissa*, and *E. brachionus* were present in the tadpole gut contents, but based on the results of the selectivity index, these species were not preferred. Rotifers are smaller than cladocerans, copepods, or ostracods; as a result, invertebrate predators, rather than vertebrates such as fish or tadpoles (Herzig, 1987), often select them. The fact that these rotifer species were present in the gut contents, available to the tadpoles, but not actively selected by them, suggests that they were incidentally ingested.

K. americana, *L. patella*, and *L. luna* were the species with the lowest availability or were not detected in the environment when the tadpoles were present. Rotifers have been previously documented as an important source of protein for tadpoles (Rossa-Feres *et al.*, 2004). Yet they also exhibit special behavior such as entering into, or remaining in, a state of diapause (Schröder, 2005), or the ability to stay at lower abundances (Walz, 1997) in the presence of predators as strategies to avoid predation, ensuring that they maintain permanence in the community.

The sharp decline in the population of *M. wierzejskii* observed in the months of September and October (Fig. 1) and the production of males and ephippial eggs can be attributed to tadpole predation of this zooplankton species (supported by the gut content analysis). Predation has been known to affect the life history and behavior of tropical cladocerans (Sarma *et al.*, 2005).

In September, *M. wierzejskii* was abundant in the aquatic system and in the gut contents of the tadpoles. Meanwhile in October, *M. wierzejskii* was absent in the ecosystem but we continued to find individuals in the gut contents of the tadpoles. In the following months, we did not find any individuals in the gut contents or in the environment.

We also observed *M. wierzejskii* with resting eggs in the gut contents of the tadpoles as well as in the environment in the month of September. Males pertaining to this cladoceran species were also present in the environment. Males and resting eggs are often only produced when the zooplankton population finds itself living under environmental stress such as lack of nutrients, competition, and predation (Slusarczyk, 1999; Lampert & Sommer, 2007).

Considering the small area of the system, and the fact that only one predator dwells within it (personal observation), we hypothesize that the tadpoles produced a profound diminishing effect on the cladoceran population. Tadpoles caused them to reproduce sexually, eventually leading to their disappearance from the artificial aquatic system, which has also been recorded in similar studies (Hamilton *et al.*, 2012; Gama-Flores *et al.*, 2013).

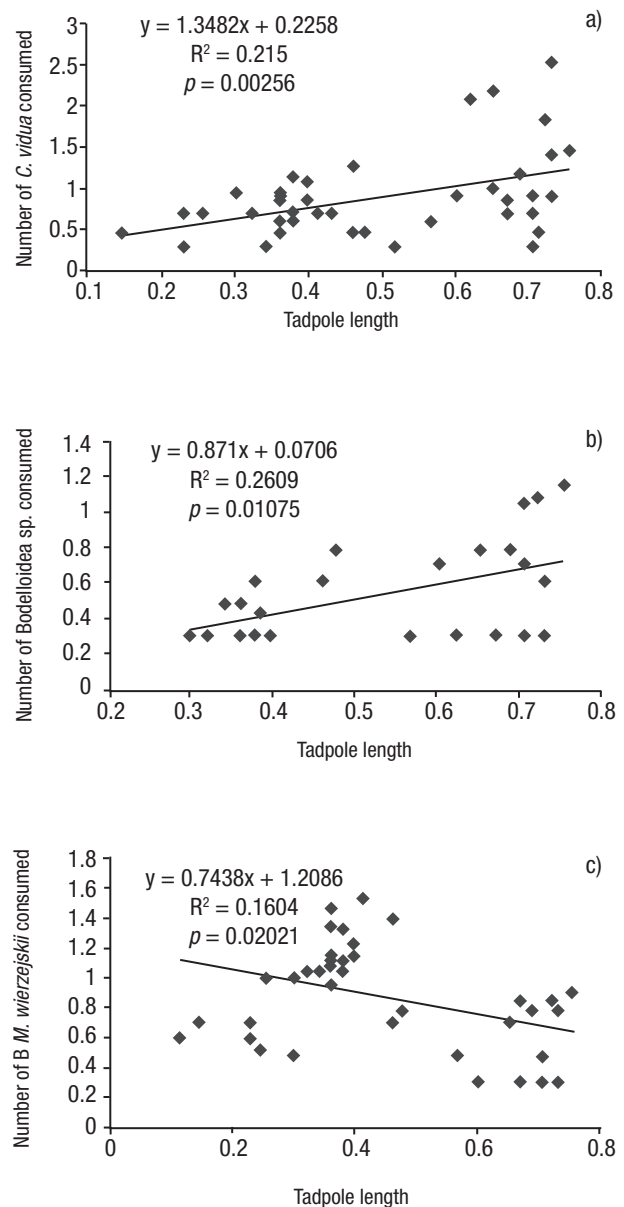
Our demonstration in this study regarding tadpole affinity for macrozooplankton, and the way in which these aquatic vertebrates influence zooplankton richness and abundance represents a great advance in understanding and defining the trophic status and dietary needs of tadpoles as well as the ecological role that they play within the aquatic systems they inhabit. This knowledge is essential in the conservation of not only *I. valliceps*, but also of other anuran species and the ecosystems in which they reside.

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Figures 4 a-c. Simple linear regression between the size of the tadpole and the quantity of zooplankton consumed in an artificial aquatic pond in Cozumel Island, Mexico: a) *Cypridopsis vidua*, b) *Bdelloidea sp.*, c) *Moina wierzejskii*.

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