OPEN ACCESS
International Journal of Aquatic Science
ISSN: 2008-8019
Vol. 7, No. 1, 25-29, 2016

IJAS

Effects of salinity and hardness on survival of *Poblana letholepis* (Atheriniformes: Atherinopsidae) larvae

Tannia Cristina Frausto Illescas; María Cecilia Hernández-Rubio* and Gerardo Figueroa-Lucero

Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n. Santo Tomás, CP 11340, D. F., México

Received: 04 November 2015 **Accepted:** 14 December 2015 **Published:** 01 January 2016

Abstract: *Poblana letholepis* is an endemic fish from crater-lake La Preciosa, Puebla, at the Mexican Plateau, Mexico. It has been a great resource for local people since pre-Hispanic times. This species is at risk of extinction due to its habitat loss in addition to the lack of knowledge about its biology. Survival and development time of *P. letholepis* were evaluated from hatching to metamorphosis to juvenile, with 20 free embryoL^{-1} reared at 0, 4, 8, 12 and 16 gL^{-1} salinities and 160-180, 80-100 and 40-48 mgL^{-1} $CaCO_3$, 20 ºC, pH 7.6 and 5 mgL^{-1} O_2, under a bifactorial design (5 x 3 levels) with three replicates per treatment. Larvae were fed with rotifers (*Brachionus plicatilis*) and *Artemia* nauplii. Treatments showed differential effects on survival (ANOVA II, $P < 0.05$) but not in juvenile metamorphosis time (50 ± 0.25 dph) (ANOVA II, $P > 0.05$). Salinity presented a significative effect on final survival, only. However, there was an interaction between these factors. The highest survival was observed at 4-8 gL^{-1} salinity and 160-180 mgL^{-1} $CaCO_3$ (80-65%) and 80-100 mgL^{-1} $CaCO_3$ (60-70%). Major survival of *P. letholepis* larvae, is probably due to physiological mechanisms such as increased excretion of sodium by chloride cells proliferation in hard water.

Keywords: species conservation, salinity, water hardness, silverside.

Introduction

Poblana letholepis Álvarez 1950 is endemic from Mexico and its unique habitat is the crater-lake La Preciosa, where it is the only vertebrate. La Preciosa lake is in the plain of lacustrine origin San Juan, Puebla, (19º22' N and 97º 23' W) at 2 333 m above sea level with 0.79 Km^2. San Juan plain belongs to endoreic basin Oriental (19°44' 00' - 18°57'00'' N and 98°02'24" - 97°09'00" W) which is extended through Puebla, Tlaxcala and Veracruz states. La Preciosa is origined from volcanic explotions which happened between the Medium tertiary and Upper Quaternary. La Preciosa shares a common history with other crater-lakes from this basin: Alchichica, Atexcac, Quechulac, Tecuitlapa and Aljojuca. These crater-lakes are named *Axalapascos* (Armienta *et al*., 2008).

Average annual temperature in La Preciosa is 17.8º C on surface while at 20 m depth is 14.9º C (Armienta *et al*., 2008). It is a water body of semi hard water (114 mgL^{-1} $CaCO_3$) and low salinity (1 gL^{-1}), pH 6.2-8.1 and 72% O_2 (Ramírez-García and Novelo, 1984; Díaz-Pardo and Guerra-Magaña, 1990; Peralta *et al*., 2002).

The silverside *P. letholepis* is in risk of extinction and the knowledge about its biology is poor. This fish is an iteroparous species with indirect development. Due to its reproductive strategy and tactics, in agreement to Balon (1985), it is an altricial species, not guarder, open substratum obligated phytophilous spawner. This species reaches a maximum length of 76.2 mm (Álvarez, 1950) and a life span about of about two years. (Díaz-Pardo and Guerra-Magaña, 1990). The aim of this study was to define the best salinity and water hardness conditions for survival juveniles to establish an adequate methodology for the conservation of this species.

Materials and Methods

P. letholepis eggs were collected from the crater-lake La Preciosa, Puebla (19°22 ' N and 97°23 ' O) on April, which is the month of maximum spawning. As others Atherinopsids, *P. letholepis* attaches eggs to roots of halophyte vegetation by a filament during

(✉) cecheru@yahoo.com.mx

natural spawning. Collected eggs were put inside plastic bags with filtered water from the crater-lake and oxygen was supplied. Plastic bags were covered with ice to reduce metabolism eggs. In the laboratory, shape, coloration, ornamentation and number of filaments eggs were examined and fifty eggs were measured.

Several batches of 50 eggs were incubated in 1 L beakers with semi hard water (100-120 mgL^{-1} $CaCO_3$), 2 gL^{-1} salinity, 20 ºC, 5 mgL^{-1} O_2, pH 7.6 and 12 h photoperiod, until free embryos hatched. The experimental design incorporated two variables (salinity and water hardness) for a total of 15 treatments (5 x 3 levels) with three replicates (n = 20) and a total n of 900 individuals (15 x 20 x 3). Each treatment was one combination of salinity (0, 4, 8, 12 and 16 gL^{-1}, Sigma ™) and water hardness (hard water (H): 160-180, semi hard water (SH): 80-100 and soft water (S): 40-48 mg L^{-1} $CaCO_3$), 20 ºC, pH 7.6, 5.5 mgL^{-1} O_2 and 12 h photoperiod. Water was replaced every day. Survival was registered each 24 h. Feeding was performed according to Figueroa-Lucero *et al.*, (2004) modified for this species. Although mixed feeding begins two days post hatching (beginning of the larval period), fish were fed *ad libitum* with the mixohaline rotifer *Brachionus plicatilis* (20 rotifers mL^{-1}) from 0 to 5 days post hatching (dph). From 6 to 35 dph, fish were fed with a combination of rotifers and *Artemia* nauplii (10 nauplii mL^{-1}). Finally, from 36 dph on until Juvenile period (J) fish were fed *ad libitum* just with *Artemia* nauplii. These food quantities were adequate to reduce larvae mortality (pers. obs.).

Survival proportion values and development time to juvenile were arcsine $\sqrt{pij}$ transformed before analysis (Montgomery, 1991). Salinity and water hardness effects on final survival and metamorphosis time were evaluated through a two-way full factorial ANOVA, $\propto$ = 0.05. Post-hov Tukey pairwise comparisons were used (p< 0.05) (Sokal and Rohlf 1995).

Results

The silverside *P. letholepis* is an iteroparous species with indirect development; its eggs are telolecithal, spherical, small (1.18 ± 0.05 mm), smooth corion, transparent, with one oil globule after division phase, and one filament.

Salinity showed a major effect in juvenile survival and it was showed an optimal type curve (ANOVA, P < 0.05) (Fig. 1); contrary, water hardness did not have a significant effect (ANOVA, P > 0.05) on final survival of juveniles. However, there was a marginally significant interaction between factors (ANOVA, P < 0.06) with regard to survival.

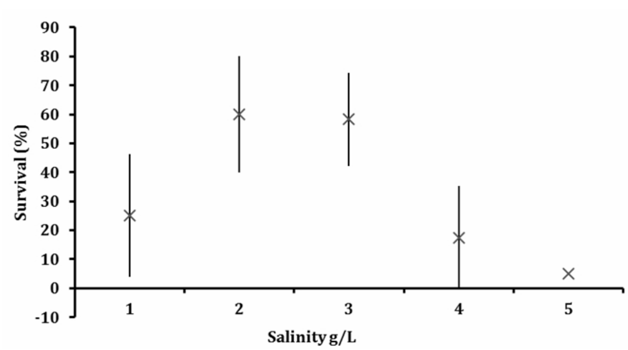

Fig. 1: Final percent survival of *P. letholepis* juveniles under salinities of 0-16 gL^{-1} after 50 dph (ANOVA, p < 0.05, three replicates per treatment).

However, in the treatments where larvae metamorphose to juveniles, the best results were obtained in hard (H) and semi hard (SH) water (ANOVA, P > 0.05) (Fig. 2).

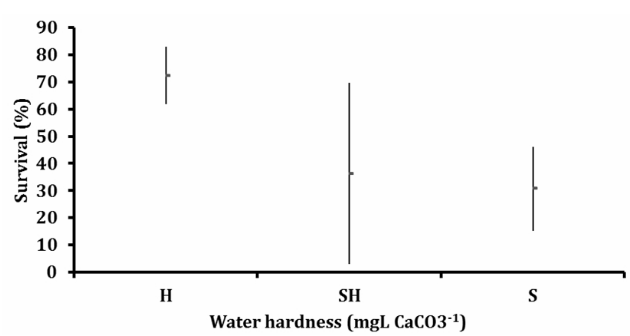

Figure 2. Final percent survival of *P. letholepis* juveniles after 50 dph under hard (H), semi hard (SH) and soft (S) water conditions (ANOVA P > 0.05, three replicates per treatment).

Larvae survival was strongly affected in the first ten dph, and the highest mortalities were at salinities up to 8 gL^{-1} in all water hardness. Larvae did not survive on H0 (11 dph), SH16 (5 dph), H12 (33 dph) and H16 (16 dph) treatments conditions. After metamorphosis of larvae, 80-60% juveniles survived in H4, SH8, H8 and SH4, in that order. Survival was 40 % in S0, S4 and S8; 30% in S12 and 10% juveniles survived in SH0, 5% survival was obtained in SH12 and S16 (ANOVA $P < 0.05$, Tukey < 0.05) (Fig. 3). Both salinity and water hardness did not have a significant effect on development time to juvenile (49-51 dph) (ANOVA, $P > 0.05$).

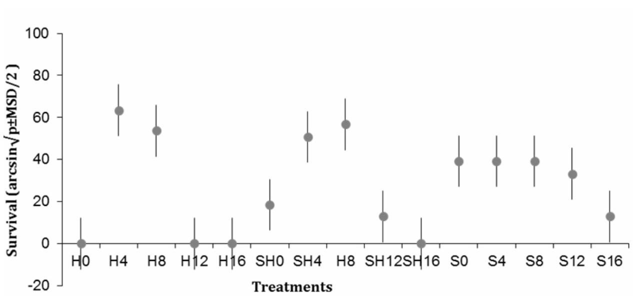

Figure 3. Survival of *P. letholepis* juveniles after 50 dph (arcsin √p ± MSD/2). (ANOVA P < 0.05, Tukey P < 0.05). Larvae did not survive on H0, H12, H16 and SH16 treatments.

Discussion

Osmoregulation is an essential process in animals, to maintain chemical and physical conditions of the body fluids (cytoplasm, plasma, extracellular fluids) within certain ranges in order to ensure body normal activities. Ions that affect more this process are Na^+ and Cl^- because they account for 90% of inorganic electrolytes. In general, fish maintain an ionic concentration similar to one third of seawater concentration, both in marine and freshwater fish (McCormick, 2001).

Survival variation of *P. letholepis* larvae is a consequence of its development type, due to the fact that adult osmoregulatory organs are under-developed or absent in the larval period. *P. letholepis* larvae showed a higher tolerance to salinity and water hardness conditions than those recorded in crater lake La Preciosa in the present study (1.5 gL^{-1} salinity and 114 mgL^{-1} $CaCO_3$ water hardness). However, hardness and other parameters can change from year to year en La Preciosa and the other crater-lakes from San Juan plain, that could explain this species wide tolerance interval to hardness and salinity (Tavera and Komarek, 1996). Several freshwater Atherinopsids species present higher tolerance to salinity in captivity than in places where they inhabit. Similar behavior has been observed in *Chirostoma humboldtianum* and *C. riojai* embryos and larvae (Hernández-Rubio, 2009; Hernández-Rubio and Figueroa-Lucero, 2013), *C. estor estor* embryos have a higher hatching percentage at 10 gL^{-1} salinity, while tolerance range is 0-10 gL^{-1} salinity in larvae (Martínez-Palacios *et al*., 2004); *C. promelas* shows the best hatching rates in 0-15 gL^{-1} and their larvae present a higher survival at 0-5 gL^{-1} (Salgado-García, 2002). 73-78% *Menidia beryllina* embryos hatching at 0-30 gL^{-1} salinity and their larvae have an optimal survival at 15 gL^{-1} and *M. peninsulae* embryos have better development at 5 gL^{-1} salinity, while larvae have higher survival percentages in 30 gL^{-1} (Middaugh *et al*., 1986). *Odonthestes bonariensis* and *O. hatcheri* larvae survived in salinities from 0 to 30 gL^{-1} with the best survival rates at intermediate salinities (10-20 gL^{-1}). Moderate salinities allow a better performance, in particular to attain stable survival rates, this suggests that salinity acts as a mitigating agent for other forms of stress (Tsuzuki *et al*., 2000). In fish almost always a better survival and growth rates is observed in intermediate salinity conditions (8-20 gL^{-1}) (Bœuf and Payan, 2001).

The capacity to respond to rapid salinity changes requires the rapid activation of existing mechanims (transport proteins and epithelia). The gills are the primary site of net sodium and chloride transport, actively taking up salts in fresh water and secreting them in sea water. Embryos and larvae regulate NaCl concentration in their bodies through chloride cells present in yolk-sac membrane of free embryos and skin of larvae, in opercular membrane principally (Kaneko & Hiroi, 2008). Salinity tolerance of *P. letholepis* larvae is a probable trait from a marine ancestor.

Studies of growth hormone (GH), insuline-like growth factor I (IGF-I) and cortisol have showed that those hormones have osmoregulatory actions and increased salinity tolerance in rainbow trout (*Onkorhynchus mykiss*) and other freshwater fish because GH and IGF-I stimulate the number and size of gill chloride cells (McCormick, 2001).

Moreover, regulation of intra and extra-cellular pH is crucial in fish, because drastic changes can alter the local charge on proteins affecting enzyme function and membrane channel properties (Brauner, 2008).

The best survival of *P. letholepis* larvae was observed at 160-180 and 100-120 mgL^{-1} $CaCO_3$. Water hardness of 40-48 mgL^{-1} is not recommended for *P. letholepis* larvae. Unfortunately, there are not experimental tests about water hardness effect in other freshwater Atherinpsids, but *C. humboldtianum* eggs have been incubated in hard water and 5 gL^{-1} salinity with high hatching percentages (Hernández-Rubio *et al.*, 2006). However, *Chirostoma* and *Poblana* species inhabit in water bodies from soft to hard water (Álvarez, 1950; Díaz-Pardo and Guerra-Magaña, 1990; Enríquez and Paulo-Maya, 1997; Paulo-Maya *et al*, 2000; Soto-Galera and Paulo-Maya, 2003).

Major survival *P. letholepis* larvae from 100-120 to 160-180 mgL^{-1} $CaCO_3$ and 4-8 gL^{-1} salinity, probably this is due to physiological mechanisms, fish have a higher tolerance to heavy metals in hard water and this could be applied to Na+ (Pascoe, 1986), such as increased excretion of sodium by chloride cells proliferation in hard water (Murad, 2013).

The wide tolerance interval to water hardness present in *P. letholepis* larvae, could be an attribute shared with the rest of the species of *Poblana* from the other Axalapascos, because each water body has different $CaCO_3$ concentrations and these conditions could have allowed *Poblana* species to colonize these lakes 5.33 millions years ago (Bloom *et al*., 2009).

Acknowledgments

The financial support was received from Secretaría de Investigación y Posgrado-IPN (SIP-20140914).

References

- ✓ Álvarez J. (1950) Contribución al conocimiento de los peces de la región de los Llanos, estado de Puebla (México). Anales de la Escuela Nacional de Ciencias Biológicas, 5: 81-107.
- ✓ Armienta A., Vilaclara G., De la Cruz-Reyna S., Ramos S., Ceniceros N., Cruz O, Aguayo A. and Arcega-Cabrera F. (2008) Water chemistry of lakes related to active and inactive Mexican volcanoes. Journal of Volcanology and Geothermal Research, 178 (2): 249-258.
- ✓ Balon E. (1986) Early life histories of fishes. New Developmental, Ecological and Evolutionary Perspectives. Dr. W. Junk Publishers, USA.
- ✓ Boeuf G. and Payan P. (2001) How should salinity influence fish growth?. Comparative biochemistry and physiology part C, 130: 411-423.
- ✓ Bloom D.D., Piller K.R., Lyons J., Mercado-Silva N. and Medina-Nava M. (2009) Systematics and biogeography of the Tribe Menidiini (Teleostomi: Atherinopsidae) based on the mitocondrial ND2 gene. Copeia, 2: 408-417.
- ✓ Brauner C.J. (2008) Acid-base balance. In: Finn and. Kapoor (eds) Fish larval physiology, Science Publishers, Enfield NH: 185-200.
- ✓ Díaz-Pardo E. and Guerra-Magaña C. (1990) Peces dulceacuícolas mexicanos II: *Poblana alchichica* (Mugiliformes : Atherinidae). Zoología Informa, 17-18: 50-57.
- ✓ Enríquez M. A. and Paulo-Maya J. (1997) Peces dulceacuícolas mexicanos XV. *Chirostoma chapalae* (Atheriniformes: Atherinidae). Zoología Informa, 35: 13-24.
- ✓ Figueroa-Lucero G., Hernández-Rubio M.C., Meza-González O.R., Arredondo-Figueroa J.L., Castro-Barrera T., Barriga-Sosa I.A. and Rodríguez C.A. (2004) Effect of food type on growth and survival of *Chirostoma riojai* Solórzano y López, 1965 (Atheriniformes: Atherinopsidae) during early development. Journal of Biological Research, 2: 93-99.
- ✓ Hernández-Rubio M.C. (2009) Historia de vida temprana de *Chirostoma humboldtianum* y *Chirostoma riojai (Atheriniformes: Atherinopsidae)* bajo la teoría de la ontogenia por saltos. Tesis Doctoral. Universidad autónoma Metropolitana, México.
- ✓ Hernández-Rubio M.C., Figueroa-Lucero G., Barriga-Sosa I.A., Arredondo-Figueroa J.L. and Castro-Barrera T. (2006) Early development of the shortfin silverside *Chirostoma*

humboldtianum (Valenciennes 1835) (Atheriniformes: Atherinopsidae). Aquaculture, 261: 1440-1446.

- Hernández-Rubio M.C. and Figueroa-Lucero G. (2012) Desarrollo temprano en peces con énfasis en el género *Chirostoma* (Atherinopsidae). In: I. A. Barriga Sosa, P. Ramírez, G. Barrera (eds). Acuicultura en México: Impacto en Producción, Manejo y Conservación, Editorial Académica Española, Deutschland: 468-536.
- Hernández-Rubio M.C., and Figueroa-Lucero G. (2013) Effects of temperature and salinity during the embryonic period of *Chirostoma humboldtianum* and *Chirostoma riojai* (Atherinopsidae) until hatching. Hidrobiológica, 23(3): 275-283.
- Kaneko T., and Hiroi J. (2008) Osmo- and Ionoregulation. In: R. N. Finn and B. G. Kapoor (eds). Fish Larval Physiology, Science Publishers Enfield, NH: 163-183.
- Martínez-Palacios C.A., Comas Morte J., Tello-Ballinas J., Toledo-Cuevas M. and Ross L.G. (2004) The effects of saline environments on survival and growth of eggs and larvae of *Chirostoma estor estor* Jordan 1880 (Pisces: Atherinidae). Aquaculture, 238: 509-522.
- McCormick S.D. (2001) Endocrine control of osmoregulation in teleost fish. American Zoology, 41: 781-794.
- Middaugh D.P., Hemmer M.J. and Rose L. (1986) Laboratory spawning cues in *Menidia beryllina* and *Menidia peninsulae* (Pisces: Atherinidae) with notes on survival and growth of larvae at different salinities. Environmental Biology of Fishes, 18(2): 107-117.
- Montgomery D.C. (1991) Design and analysis of experiments. 5th ed. New York: John Wiley & Sons.
- Murad A.K. (2013) Effects of humic acid and water hardness on acute cadmium toxicity by using *Simocephalus exspinosus* as a bioindicater. Euphrates Journal of Agricultural Science, 5(4): 17-25.
- Paulo-Maya J., Figueroa-Lucero G. and Soria-Barreto M. (2000) Peces dulceacuícolas mexicanos XIX. *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae). Zoología Informa 43: 59-74.
- Pascoe, D., S. Evans A. and Woodworth, J. (1986) Heavy metal toxicity to fish and the influence of water hardness. Archives of Environmental Contamination and Toxicology, 15(5): 481-487.
- Peralta L., Escobar E., Alcocer J. and Lugo A. (2002) Oligochaetes from six tropical crater lakes in Central Mexico: species composition, density and biomass. Hydrobiologia, 467: 109–116.
- Ramírez-García P. and Vázquez-Gutiérrez F. (1989) Contribución al estudio limno botánico de la zona litoral de seis lagos cráter del estado de Puebla. Anales del Instituto de Ciencias del Mar y Limnología, 16: 1-16.
- Salgado-García R.L., Martínez-Palacios C.A., Racotta-Dimitrov I.S., Campos-Mendoza A. and Ross L.G. (2006) El efecto de la salinidad en larvas y juveniles de Pez Blanco de chapala *Chirostoma promelas*. III Encuentro Participación de la Mujer en la Ciencia, México.
- Sokal R.R. and Rohlf F.J. (1995) Biometry. 3rd ed. Freeman and Co., New York.
- Soto-Galera E. and Paulo-Maya J. (2003). Peces dulceacuícolas mexicanos XX. *Chirostoma jordani* Woolman 1894 (Atheriniformes: Atherinopsidae). Zoología Informa 44: 37-54.
- Tavera R. and Komárek J. (1996) Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla State, Mexico. Algological Studies, 83: 511-538.
- Tsuzuki M.Y., Aikawa H., Strüssman C.A. and Takashima F. (2000) Comparative survival and growth of embryos, larvae, and juveniles of pejerrey *Odontesthes bonariensis* and *O. hatcheri* at different salinities. Journal of Applied Ichthyology, 16: 126-130.