Tolerance and Mortality of *Gambusia affinis* to Acute Ammonia and Nitrite Exposure at Various Salinities

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ABSTRACT

Tolerance and mortality of mosquito fish, *Gambusia affinis* to acute ammonia and nitrite exposure at various salinities were studied in fibreglass tanks (500L) for a period of 4 months at KAU Fish Farm, Faculty of Marine Sciences, Jeddah. Salinity tolerance was tested in 0 (Control) 10, 20, 30 and 40 ppt. After tolerance study, 60 fish from each salinity was taken for ammonia and nitrite exposure study. Ammonia toxicity was tested in 0, 0.5, 1, 2, 3 and 4ppm; while nitrite was tested at 8, 10, 12, 14 and 16 ppm. Survival decreased when salinity increased from 0 to 40 ppt and a significant relationship (R^2=0.959) was found between increase in salinity and survival. Mortality rate was found to be increased when ammonia and salinity level increased and the highest mortality was observed in the highest dosage of ammonia (4ppm) and salinity (40ppt). Similarly, increased mortality was observed in higher levels of nitrite and salinity. Results show that *Gambusia affinis* can tolerate salinity up to 40 ppt, ammonia at 1ppm and nitrite toxicity at <8ppm.

تحمل أسماك Gambusiaaffinisإلى التعرض الحاد للأمونيا والنيتريت في درجات مختلفة من الملوحة

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المُستلخص

أجريت تجارب على تحمل أسماك mosquito و *Gambusiaaffinis* للتعرض لتراكيز الحاده من الأمونيا والنيتريت في درجات الملوحة المختلفة. تم إجراء التجارب في أحواض من الفيبرجلاس سعة (500 لتر) لمدة 4 اشهر في المزرعة اسمكية لكلية علوم البحار, بأبحر. تم إختبار تحمل الأسماك للملوحة عند كلا من 0 (الضابط), 10, 20, 30, 40 جزء في الألف. حينما تمت أقلمة الأسماك لدرجات الملوحه 0, 10, 20, 30, 40 جزء في الألف, تم جمع عدد 60 من الأسماك من كل درجة ملوحة ومن ثم دراسة تعرضها للأمونيا والنيتريت. تم إختبار سمية الأمونيا عند التراكيز 0, 0.5 , 1, 2, 3 و 4 جزء في المليون في حين تم إختبار سمية النيتريت عند التراكيز 8, 10, 12, 14و 16جزء في المليون. لوحظ إنخفاض في نسبة البقاء على قيد الحياه عند زيادة الملوحة من صفر إلى 40 جزء في الألف وكانت العلاقة بين الملوحة ونسبة البقاء ذات دلاله إحصائية معنوية (R2=0.959). كما لوحظ زيادة معدل الوفيات عند زيادة جرعات الأمونيا وإرتفاع مستوى الملوحه ولوحظ بأن أعلى معدل للوفيات كان عند أعلى جرعة من الأمونيا (4 جزء في المليون) وملوحة (40 جزء في الألف). وقد لوحظ بالمثل زيادة معدلات الوفيات في المستويات الأعلى من النيتريت والملوحة. وتظهر النتائج بأن *Gambusiaaffinis*يمكن أن تتحمل درجات الملوحة حتى 40 جزء في الألف ومستويات أقل سمية من الأمونيا والنيتريت.

Introduction

The mosquito fish, *Gambusia affinis* is a fresh water fish species introduced to many parts of the world as a biocontrol to lower mosquito populations (Benoit,*et al*., 2000; Al-Hafedh, 2007). The fish can live in a wide variety of water types, being very tolerant to high water temperatures, a wide range of salinities, pH and very low dissolved oxygen levels (Peterson and Peterson, 1990). For these reasons, this species may now be the most widespread fresh water fish in the world, having been introduced as a biocontrol to tropical and temperate countries in both hemispheres and then spread further both naturally and through even further introductions.

Environmental salinity has been demonstrated to influence NH_3-N and NO_2-N toxicity in several fish species (Sampaio, *et al*., 2002., Crawford and Allen, 1977; Alabaster,*et al*., 1979). Lee,*et al*. (2005) studied the adaptability of juvenile Fugu, *Takifugu rubripes* to low-salinity environments and found that the fish lower limit of salinity tolerance lies between 5 and 10% seawater. Even though there are good work on salinity tolerance in fish species, information on salinity tolerance of *G.affinis* is scanty.

In recent years, the harmful effects of nitrite on fish have attracted much attention (Das,*et al*., 2004; Voslarova*, et al*., 2008). Elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments and in effluents from industries producing metal, dyes, and celluloid (Pitter, 1999). Increased nitrite concentrations in water are also one among the most frequent problems encountered both in aquariums and in fish farms (Adamsson, *et al*., 1998; Etscheidt, 2003; Dvorak, 2004; Svobodova, *et al*., 2005).Nitrite (NO2) naturally occurs in fresh water and is an intermediate product in bacterial nitrification and denitrification processes in the nitrogen cycle. In oxygenated waters, nitrite is transformed by nitrification to nitrate and in anoxic conditions the elementary nitrogen is the product of biological denitrification (Pitter, 1999). Literature dealing with the long-term toxicity of sub lethal nitrite concentrations corresponding to 10 % of 96 h LC50 suggests that such concentration should not be detrimental to freshwater fish (Wedemeyer and Yasutake, 1978). Neither growth suppression nor tissue damage was observed (Colt,*et al*., 1981). Acclimatisation of fish to elevated nitrite concentrations was studied by Tucker and Schwedler, (1983); Doblander and Lackner, (1997) and Machova *et al*. (2004). Acute toxicity of nitrite has been investigated and described in a number of fish species (Bowser, *et al*., 1983; Crawford and Allen, 1977; Russo and Thurston, 1977, McConnell, 1985, Pistekova, *et al*., 2005, Kroupova, *et al*., 2006; Voslarova, *et al*., 2006) but the effects of chronic nitrite exposure on their survival and growth have not been well documented yet.

Ammonia is one of the most pervasive contaminants in aquatic habitats and it is contributed to aquatic ecosystems through multiple sources including natural biogenic and anthropogenic sources. Ammonia primarily exists in two forms: NH_3 (un-ionized ammonia) and NH_4^+ (ammonium ion). Unionized ammonia is the more toxic form and increases in proportion as pH increases above 8.0 and can induce numerous effects in fish including loss of equilibrium, gill hyperplasia, and mortality due to the lack of an outward diffusion gradient across the gill surface in high aqueous concentrations (Le- Ruyet, *et al*., 1997; Soderberg, *et al*., 1984). However, no study has hitherto been conducted to understand the tolerance of *G. affinis* to acute ammonia and nitrite exposure atvarious salinities and therefore, a study wasconducted to evaluate the tolerance of *Gambusia affinis*to acute ammonia and nitrite exposure atvarious salinities.

1. Materials and methods

1.1 Experimental set up

The study was conducted at the University Fish Farm, Faculty of Marine Sciences, King Abdul Aziz University campus at Obhur, Jeddah for a period of 6 months. Required number of *G. affinis* was brought from Al-Ihsaa city, Saudi Arabia and stocked in a fiber glass tank (1000 L) containing fresh water for acclimation to laboratory conditions. During acclimation, fishes were fed with Aquafin flake feed (Aquafin, China) at three intervals (8am, 1pm and 7pm) daily at the rate of 5% body weight. After one week of acclimation, fishes were transferred for salinity tolerance trial.

1.2. Salinity tolerance study

Salinity tolerance of fish was tested in0 (Control) 10, 20, 30 and 40 ppt. For this, 5 fibreglass tanks (500 L) were taken and each tank labelled as 0, 10, 20, 30 and 40 ppt and filled with fresh water prior to experiment. All tanks were provided with biological filter, adequate aeration and stocked with 400 pieces of uniform size (4.4 ±1.1 cm; 2.4 ± 1.2 g) *G.affinis*. Salinity in each tank was increased at 2ppt/twice a day and stopped when the tank water reached the respective salinity at10, 20, 30 and 40ppt. Once the fishes reached and adapted to the respective salinity level,they were collected for acute ammonia and nitrite exposure study. Water quality parameters such as dissolved oxygen, pH, temperature and salinity in each tank were monitored daily. Fishes were fed with Aquafin flake feed at the rate of 5% of the body weight/day at8am, 1pm and 7pm.

1.3. Ammonia and Nitrite toxicity study

The study was conducted in parallel with salinity tolerance study. Once the fishes were acclimatized to 0, 10, 20, 30 and 40 ppt salinity, sixty fish from each salinity was collected and used for ammonia and nitrite toxicity study. Toxicity of ammonia was tested in 0, 0.5, 1, 2, 3 and 4 ppm and nitrite in 8, 10, 12, 14 and 16 ppm. For this, glass beakers (3 L) were filled with required dose of Ammonia and Nitrate separately and each test was triplicated. Six fishes were stocked in each beaker and the mortality of fish was recorded every three hours till 96 hours of exposure. Feeding was not done during this period.

1.4. Statistical Analysis

One way analysis of variance (ANOVA) was employed to find out the statistical difference in tolerance and mortality of fishes between different concentrations of salinity, ammonia and nitrite (Snedecor and Cochran, 1989). Duncan's multiple range test was applied to find out the statistical difference between treatment means (Steel and Torrie, 1980). Linear regression analysis was also employed to find out the relationship between salinity tolerance and mortality of fishes.

2. Results

2.1. Water quality parameters

Details on water quality parameters recorded during salinity tolerance study (30 days) are depicted in Fig. 1. ANOVA showed no significant difference ($p > 0.05$) in water temperature, dissolved oxygen and pH between control and treatment tanks and these parameters were found to be within the tolerable range for the fish. Survival (%) of fishes recorded during salinity tolerance study is shown in Table 1. Reduction in survival was noticed when salinity increased from lower (0 ppt) to higher level (40 ppt). Linear relationship shows that there was significant relationship (R^2=0.9592) between salinity and survival (Fig. 2). In increase in salinity, survival was found to be decreased.

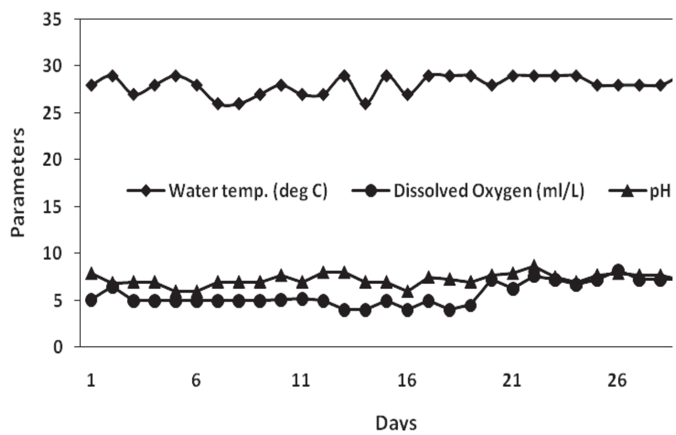

Fig. 1. Water quality parameters recorded during salinity tolerance study

Table 1- Survival (%) of fishes in salinity tolerance study

Parameters	Control (0ppt)	10ppt	20ppt	30ppt	40ppt
Initial numbers	400	400	400	400	400
Final numbers**	389[a]	370[a]	358[b]	332[c]	321[d]
Survival (%)	95	93	90	83	80

** $p<0.01$; a,b,c,d: Means with the same superscript do not differ from each other (Duncan's test)

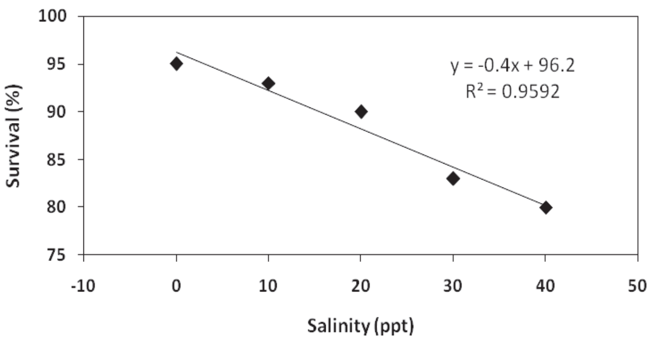

Fig. 2. Linear relationship between salinity and survival

2.2. Ammonia toxicity study

Details on mortality of fishes treated indifferent concentrations of ammonia under various salinity are presented in Table 2. Mortality rate increased when ammonia and salinity increased and high mortality rate was observed in higher concentrations of ammonia (4ppm) and salinity (40ppt). Fishes treated with ammonia at 0.5ppm showed 100% tolerance for 72 hours in salinity from 0 to 30ppt and it was significantly ($p<0.01$) differed from other concentrations of ammonia and salinity. Moreover, fishes were able to tolerate ammonia up to 4ppm for 48 hours in 0ppt

Table 2. Mortality (%) of fishes in different concentrations ofammonia and salinity.

Salinity (ppt)	Exposure (Hours)	Ammonia (ppm)					
		0	0.5*	1*	2*	3*	4*
0*	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	17	17
	96	0	0	17	17	50	83
10*	24	0	0	0	0	0	0
	48	0	0	0	0	17	17
	72	0	0	0	34	50	50
	96	0	0	34	50	100	100
20*	24	0	0	0	0	0	0
	48	0	0	0	0	17	17
	72	0	0	17	34	50	64
	96	0	0	34	50	100	100
30*	24	0	0	0	0	0	0
	48	0	0	0	0	17	17
	72	0	0	17	34	64	64
	96	0	17	34	50	100	100
40*	24	0	0	0	0	0	0
	48	0	0	0	0	17	17
	72	0	17	50	50	64	83
	96	0	34	64	83	100	100

* $p<0.01$

2.3. Nitrite toxicity study

Mortality of *G. affinis* treated in different concentrations of nitrite and salinity are shown in Table 3. Mortality rate increased when nitrite and salinity increased and complete mortality was occurred from 72 and 96 hours of exposure. Fishes treated with Nitrite at 8ppm showed 100% tolerance for 24 hours in all salinity concentrations and it was significantly ($p<0.01$) differed from other concentrations of nitrite and salinity

Table 3. Mortality (%) of fishes in different concentrations of nitrite and salinity.

Salinity (ppt)	Exposure (Hours)	Ammonia (ppm)					
		0	8*	10*	12*	14*	16*
0*	24	0	0	17	33	33	50
	48	0	17	33	66	83	100
	72	0	100	100	100	100	100
	96	0	100	100	100	100	100
10*	24	0	0	17	33	33	66
	48	0	33	50	66	100	100
	72	0	100	100	100	100	100
	96	0	100	100	100	100	100
20*	24	0	0	33	33	50	83
	48	0	50	50	66	100	100
	72	0	100	100	100	100	100
	96	0	100	100	100	100	100
30*	24	0	0	17	17	66	83
	48	0	50	66	83	100	100
	72	0	100	100	100	100	100
	96	0	100	100	100	100	100
40*	24	0	0	17	33	100	100
	48	0	50	83	83	100	100
	72	0	100	100	100	100	100
	96	0	100	100	100	100	100

*$p<0.01$

3. Discussion

Result of the study shows that *G. affinis* can tolerate salinity up to 40ppt (sea water) by gradual increase in salinity at the rate of 2 ppt/day. Chervinski (1983) and Nordlie and Mirandi (1996) reported that *G. holbrooki* and *G. affinis* could tolerate direct transfer to 19.5% salinity and that 65% of the fishes survived in sea water, while 50% survived in water at 58.5% salinity for 7 days. Salinity adaptation by euryhaline teleosts is a complex process involving a suite of physiological and behavioural responses to environments with differing osmoregulatory requirements (Swanson, 1998). Salinity is expected to affect metabolism through changes in energy expenditure for osmoregulation and energy costs are expected to increase in water that deviates from the isosmotic level (Swanson, 1998).These results clearly indicate that *G. affinis* is a euryhaline fish and it can tolerate and survive in high levels of salinity.

The metabolic costs of osmoregulation are significant, and animals exposed to conditions outside of their normal operating levels, such as hyper salinity, may be adversely impacted by the stress and additional energy required by osmoregulatory processes. In general, fish species span a range of potential salinity tolerance, which often does not correlate to the natural salinity exposure undergone by each species. Thus, the ability of fishes to transition between the two distinct regulatory systems characteristic of teleosts in either seawater or freshwater is an essential trait, and study of this transition allows for better understanding of each system and its most essential components for water and ion transport. Results of the present study show that there is a significant relationship between survival and salinity and survival decreases with increase in salinity level. This may be due to the stress in osmoregulation as observed in the case of *O.beta* (McDonald and Grosell, 2006).

Result of ammonia toxicity study reveals that ammonia is stressful to *G. affinis* at high concentrations. It also shows that mortality is related to increasing levels of ammonia, salinity and exposure time. Highest mortality (100%) was observed in higher dose (3 and 4ppm) of ammonia and longer exposure (96 hours). Fishes in these concentrations were found to be under acute stress. This may be due to the result of the increased activity of glutamine synthetase, a detoxification mechanism as suggested by Mommsen and Walsh (1992). Glutamine synthetase mediates the amination of glutamate and NH_3 in the formation of glutamine. While excess activity through this pathway consumes vital brain ATP and glutamate resources and can result in neurotransmission failure, the severity of this effect varies with the degree of ammonia exposure (Mommsen and Walsh, 1992). Madison *et al*., (2009) propose that when fishes exposed to moderate levels of ammonia, fish may be "calmed" by this mechanism through reduced release of stress induced compounds. Person-Le Ruyet *et al*., (1998) have shown that ammonia entered fish within 15 minutes of exposure. The observed effects on the changes in behavior were similar to those reviewed by Haywood (1983). They were associated with erratic actuations on self-feeders and with an increase in the oxygen consumption. These feeder actuations were not a consequence of feeding activity, but of abnormal swimming at the surface. These were the result of the buildup of ammonia in the blood and other tissues (brain, liver, muscle) which has negative effects on synaptic connections of the central nervous system and the NMDA (N-Methyl-D-aspartic Acid) receptor activity (Russo and Thurston, 1991; Tomasso, 1994; Montfort, 2000).

Teleost fishes could tolerate low concentrations of ammonia and salinity for short duration of exposures (Okelsrud and Pearson, 2005). In the present study, *G. affinis* was able to tolerate all levels of ammonia and salinity concentrations when it was subjected for 24 hours of exposure, which indicates that the fish can tolerate and overcome adverse environmental conditions of ammonia and salinity for a short period. The high mortality observed in higher concentrations of ammonia and salinity may be due to the combined effect of ammonia and salinity. Similar high mortality was also observed in juveniles of barramundi when exposed to 2 and 2.5mg/liter of ammonia concentrations (Okelsrud and Pearson, 2005).

Solbe and Shurben (1989) reported early life stages of rainbow trout (*O.mykiss*) are considerably more sensitive to ammonia toxicity than older stages, while Mallet and Sims (1994) reported newly hatched larvae and early fry are more sensitive to ammonia exposure than older fish and eggs of both carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*). Apparently there is considerable variation between species; however, according to Rand and Petrocelli (1985), smaller individuals are generally more sensitive to toxicants because of their higher surface- to volume ratio, which promotes higher relative intake of the tested chemical. Currently no information exists on the effects of ammonia toxicity to different sizes of mosquito fish, *G.affinis*. In the present study healthy adults fishes of uniform size (2.4g) from both sexes were selected and they could tolerate low levels of ammonia concentrations.The experiment revealed that higher concentrations of nitrite are detrimental to mosquito fish, *G. affinis* and mortality is correlated to increasing levels of nitrite concentrations combined with increasing salinity and exposure time. However, in low concentration at 8ppm, *G affinis* was able to tolerate nitrite toxicity in all salinity level. The mode of action of nitrite in fishes is that it diffuses into red blood cells, where it oxidizes haemoglobin to methaemoglobin with a subsequent reduction of oxygen-carrying capacity and oxygen affinity (Cameron, 1971; Jensen *et al*., 1987; Williams *et al*.,1993). Nitrite is an intermediate in the oxidation of ammonium to nitrate as it changes haemoglobin to methemoglobin, which does not carry oxygen; nitrite may thus cause anoxia in fish and other aquatic organisms. This may be the reason for mortality observed in higher nitrite concentrations.

Doblander and Lackner (1997) and Machova *et al*., (2004) suggested that fish are able to acclimate to nitrite, but the nature of the mechanism underlying this adaptation is unclear. Tucker and Schwedler (1983) demonstrated that channel cat fish could toleratea low nitrite level which is in agreement with the results of our study. Ze-quan*et al.*, 2009 studied the acute toxicity of ammonia and nitrite to mosquito fish (*Gambusia affinis*) in polluted rivers and suggested that *G. affinis* was the dominant species which could tolerate higher levels of ammonia and lower levels of nitrite. Results of the present study indicate that *G. affinis* could tolerate lower concentrations of ammonia and nitrite when exposed for a short duration of 24 hours and mortality begins from 48 hours of exposure onwards. It is also suggested that fishes could tolerate a wide range of salinity variation from fresh water to sea water. It can be acclimatized to seawater (40ppt) by gradual increase of salinity of 2 ppt per day which shows the remarkable capacity of salinity tolerance.

Conclusion

G. affinis can tolerate salinity up to 40ppt if a gradual increment of 2ppt/day is given from fresh to sea water. There was significant relationship between survival and salinity, and survival decreased with increase in salinity. Ammonia was found to be detrimental to *G.affinis* and it could tolerate 3ppm ammonia for a period of 48 hours exposure in freshwater (0ppt). Mortality rate increased with increase in salinity, ammonia and exposure time. Linear relationship shows that mortality rate increases with increasing ammonia and salinity. This was due to the synergistic effect of ammonia and salinity. Nitrite was found to be more sensitive toxic compound than ammonia. High mortality was also observed in high concentration of nitrite and salinity. Linear relationship shows that mortality rate increases with increasing levels of nitrite. It is recommended that *G. affinis* can be effectively used for the control of mosquito population in saline and stagnant water bodies where ammonia and nitrite concentrations are low.

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