Preliminary Investigations into the Control of Speckled Livebearers (*Phalloceros caudimaculatus*)

A report to Primary Industries and Resources South Australia - Biosecurity

Dale McNeil, Simon Westergaard and Dean Hartwell

SARDI Publication No. F2010/000306-1
SARDI Research Report Series No. 452

ISBN: 978-1-921563-32-4

SARDI Aquatic Sciences
PO Box 120 Henley Beach SA 5022

April 2010

Government of South Australia
Biosecurity SA

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Preliminary Investigations into the Control of Speckled Livebearers (*Phalloceros caudimaculatus*)

A report to Primary Industries and Resources South Australia - Biosecurity

Dale McNeil, Simon Westergaard and Dean Hartwell

SARDI Publication No. F2010/000306-1
SARDI Research Report Series No. 452

ISBN: 978-1-921563-32-4

April 2010

This publication may be cited as:
McNeil, D.G., Westergaard, S. and Hartwell, D (2010). Preliminary investigations into the control of speckled livebearers (*Phalloceros caudimaculatus*). Report to Primary Industries and Resources South Australia - Biosecurity. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2010/000306-1. SARDI Research Report Series No. 452. 29p.

South Australian Research and Development Institute
SARDI Aquatic Sciences
2 Hamra Avenue
West Beach SA 5024

Telephone: (08) 8207 5400
Facsimile: (08) 8207 5481
http://www.sardi.sa.gov.au

Cover photo: Speckled Livebearers (*Phalloceros caudimaculatus*) –Simon Westergaard

DISCLAIMER
The authors warrant that they have taken all reasonable care in producing this report. The report has been through the SARDI Aquatic Sciences internal review process, and has been formally approved for release by the Chief, Aquatic Sciences. Although all reasonable efforts have been made to ensure quality, SARDI Aquatic Sciences does not warrant that the information in this report is free from errors or omissions. SARDI Aquatic Sciences does not accept any liability for the contents of this report or for any consequences arising from its use or reliance placed upon it.

Printed in Adelaide: June 2010

SARDI Publication No. F2010/000306-1
SARDI Research Report Series No. 452

ISBN: 978-1-921563-32-4

Author(s):	Dale McNeil, Simon Westergaard and Dean Hartwell.
Reviewer(s):	Josh Fredburg and Leith Thwaites
Approved by:	Dr Qifeng Ye Program Leader – Inland Waters & Catchment Ecology
Signed:	
Date:	24 June 2010
Distribution:	PIRSA - Biosecurity, SAASC Library, University of Adelaide Library, Parliamentary Library, State Library and National Library.
Circulation:	Public Domain

ACKNOWLEDGEMENTS

This work was conducted on behalf of the South Australian Department of Primary Industries and Resources (PIRSA) Biosecurity Section by the South Australian Research and Development Institute (SARDI) Aquatic Sciences. The authors would like to thank John Gilliland from PIRSA Biosecurity for managing the project and providing research direction as well as directing field operations and undertaking various key steps in preparation for the field application. Thanks also to the project steering committee, especially Vic Neverauskas and Heidi Alleway (PIRSA), Pedro Schultz (AMLRNRMB), and Peter Goonan (EPA) for assisting in the development of the project scope and methods and approach. David Fleer and Cathleen Ye assisted with collection of fish and water testing in the field. Chris Story and Kendon Chemicals kindly supplied rotenone in liquid preparation. Qifeng Ye at SARDI provided program and staff management for SARDI Inland Waters and Catchment Ecology and contributed to project steering groups.

Table of Contents

EXECUTIVE SUMMARY

In 2008, a large population of the speckled livebearer (*Phalloceros caudimaculatus)* were discovered in upper Willunga Creek in the southern suburbs of Adelaide, South Australia. Initial surveys revealed that no native fish species were present in the creek and an initial research report into the population outlined a number of recommendations for assisting in the control of the pest population, including the use of rotenone or other chemical control agents, as well as the eventual re-introduction of native fishes to the creek to maintain competitive or predatory pressure on the livebearer population, if eradication proved unachievable (McNeil et al 2008). In 2009, the project steering committee recommended a series of laboratory investigations to determine the following:

1: The tolerance of speckled livebearer to potential control agents, specifically rotenone and salt.

2: The nature of competitive and predatory interactions between speckled livebearer and local native fishes, e.g. mountain galaxias (*Galaxias olidus*) and congolli (*Pseudaphritis urvilii*).

3: A trial application of rotenone as a control mechanism was also commissioned focussing on a small area of the creek located in the upper extent of the catchment that is likely to have been the historical release point of the species, as it's upstream of a significant barrier to upstream colonisation.

Laboratory investigations identified that speckled livebearers were exceptionally tolerant of salinity levels approximating that of sea-water, with direct transfer LC_{50} values of 24.7 ppt and slow acclimation LC_{50} of 38.4 ppt salinity. Pulsing salinity is therefore not considered a viable option for control of this species in freshwater systems. Rotenone applications proved far more useful as a control agent with LC_{50} values of 0.63ppm using liquid product and 0.30ppm using powder. These LC_{50} values however, exceed the concentrations approved for use under the APVMA permits that prescribe rotenone dosage for freshwater fish control, suggesting that this species is exceedingly tolerant to rotenone.

The field trial proved extremely useful for developing methods, protocols and equipment for the application of rotenone for eradication purposes. Large numbers of speckled livebearer were removed following rotenone treatment at the recommended 0.25ppm dosage using powdered rotenone. Follow-up surveys, however, revealed that the eradication trial was only successful in removing around half of the population (relative abundance). In line with the results of the

laboratory trials, it is therefore recommended that higher dosage rates of rotenone should be trialled, given the tolerant nature of the species.

Interaction trials revealed that native fishes, in particular Galaxias and congolli, will prey upon smaller speckled livebearers. However, our results indicate that livebearers out-compete smaller Galaxias for food resources. Large congolli were found to eat significant numbers of livebearers, even in the presence of alternative food sources, during initial stages of the interactive trials. After approximately five days, congolli ate very few livebearers, preferring the alternative (non-live) food sources.

These results show that the introduction of congolli may serve as an additional ecological pressure on the livebearer population but that, in the presence of alternative food sources, congolli are unlikely to serve as an eradication option. Overall, the re-introduction of native Galaxias and congolli to the reach is recommended as part of the long term strategy for livebearer control. Finally, rotenone levels were found to deteriorate rapidly following application, falling below toxic levels within 24h and subsequently remaining at very low levels (0.05ppm) following the trial.

1 INTRODUCTION AND BACKGROUND

In 2008, reports of an aquarium fish present in Willunga Creek, south of Adelaide, resulted in the discovery of a well established and self-sustaining population of speckled livebearer (*Phalloceros caudimaculatus)* (McNeil & Wilson 2008). Subsequent surveys found no native fish were present in Willunga Creek; however, a population of native mountain galaxias (*Galaxias olidus)* were present in the adjacent Wirra Creek, where the speckled livebearer was absent.

The speckled livebearer is a highly competitive invasive species that can take advantage of low-quality dietary items (such as detritus) not typically preferred by other fish species; further, it is capable of recruiting year round in certain climates (Maddern 2005). The species is hardy, easy to transport and breeds prolifically, with one female fish capable of carrying 20-80 young (McDowell 2000). Speckled livebearers have established similar populations in other Australian states and have been the subject of targeted research and control programs in Western Australia (Maddern 2005) and New South Wales (Rayner & Creese 2006).

The hardy and prolific nature of livebearers and the ease of translocation raise concerns that Willunga Creek could be an entry point for more widespread invasion. Koehn (2004) highlighted the risks posed to freshwater communities where invasive species spread, establish, and dominate fish communities as a result of inaction at early stages of infestation. This emphasises the urgent need for control measures to contain and eliminate the current population in Willunga Creek and to prevent the spread of the fish to surrounding catchments such as the Onkaparinga River, which would preclude eradication.

To address these concerns, PIRSA-Biosecurity commissioned a program of preliminary research into the status of the fish population (McNeil & Wilson 2008) as well as some preliminary investigations to inform potential control options, which is the focus of the current report. Preliminary investigations focussed on the tolerance of speckled livebearer to possible control treatments, specifically the ichthyocide rotenone and salt (to which this freshwater species may be particularly sensitive).

A second series of experiments examined ecological (competitive and predatory) interactions between speckled livebearer and local native fishes, specifically the mountain Galaxias (*Galaxias olidus*) and Congolli (*Pseudaphritis urvilii*), to ascertain the potential for restocking of these species to contribute to livebearer control programs. Finally, a field trial of rotenone application was commissioned to develop procedures and methods for control programs and to confirm the laboratory tolerance results.

2 COLLECTION OF EXPERIMENTAL FISH

Speckled livebearers were obtained for experimentation through fyke netting (3mm mesh, 3m single wing) in Willunga creek on the 29th of June and again on the 8th of Sept 2009. Mountain Galaxias were collected using a backpack electro-fisher (Smith-Root LR-24) and 4m seine (3mm mesh) in nearby Brownhill Creek in November 2009. Congolli were collected from the Lower Murray River adjacent to the Goolwa barrages in August 2009 using fyke nets (3mm mesh, 3m single wing).

Fish collected from the wild were transferred in aerated buckets to a controlled environment room (CER) at SARDI Aquatic Sciences, West Beach, South Australia, and allowed to acclimate to the temperature and water quality in the CER over 2 hours by the gradual addition of filtered bore water before placement into a 300L tank. Fish were held for the duration of the experiments in the CER which was maintained at 14-22°C, giving a water temperature of around 15-22°C.

3 SALINITY MANIPULATION TRIALS

3.1 Methods

3.1.1 Direct transfer toxicity tests

Two methods, direct transfer and gradual acclimation trials, were used to assess the tolerance of the speckled livebearer to salinity. The method used for both direct transfer and gradual acclimation followed Williams and Williams (1991). Direct Transfer trials used experimental aquaria of 18L maintained at 23°C, with a small foam filter supplied to each tank to maintain oxygen levels, prevent stratification, and filter the water. Photoperiod was controlled at approximately 12:12 light dark ratio.

In direct transfer experiments, fish were placed directly from freshwater into a series of aquaria over a predetermined gradient of salinity conditions (0.0, 4.5, 7.6, 12.6, 21 and 35ppt). Salinities were set independently using seawater within each tank to the concentration required for each treatment. These represent a logarithmic gradient in ambient salinity from fresh water to approximately seawater as per methods in Green (1965). This gradient was replicated three times for a total of eighteen aquaria (*n*=10 fish per aquarium). Experimental fish varied in size (Appendix

1) and were randomly assigned to each aquarium and the number of mortalities recorded after 1, 3, 6 and 12 h, then daily for seven days at 24, 48, 72, 96, 120, 144 and 168 h from trial initiation.

Water quality parameters were monitored daily and small adjustments to the salinity level were made with the aim of maintaining salinity to within 0.1ppt of target salinity. Additional water quality parameters measured were dissolved oxygen (DO), total dissolved solids, pH and temperature. At the start and completion of each trial, ammonia and nitrite concentrations were measured with Aquarium Pharmaceuticals® test strips. Mortalities and observations of condition, including behavior of the fish, were recorded daily and subjectively assessed, noting indications of stress including dark coloration, reduced appetite, and erratic swimming behavior or loss of equilibrium before adjusting salinity to target levels.

3.1.2 Slow Acclimation Tolerance trials

Slow acclimation trials were conducted on speckled livebearers of various sizes (Appendix 2). Six 20L aquaria were used for the slow acclimation trials, three of which were maintained as a control (remaining at ~1ppt salinity) and three were subjected to a gradual increase in salinity at a rate of 2ppt/day. Salinity was initially raised by the addition of filtered seawater; however, concentrations above sea water were obtained with the use of Ocean Nature® salt mixed with filtered seawater. Ten speckled livebearers were introduced into each of the control and treatment aquaria allowing two weeks for acclimation to the tank conditions at a constant temperature of approximately 23°C and a light: dark ratio approximating 12:12h. Fish were maintained on a diet of commercial aquaculture pellets.

Control and test fish were subject to identical diet and environmental conditions with the exception of increased salinity. Each day the number of mortalities was recorded from both control and treatment tanks as was the presence of disease or health conditions, behavior changes - including number of fish actively swimming, gill ventilation rate (GVR; opercula beats per minute) and the presence of any disturbances in the fishes equilibrium (i.e. maintaining balanced position in the water). Daily monitoring of water quality (dissolved oxygen, total dissolved solids (as a measure of salinity), pH and temperature) was undertaken to ensure adequate water chemistry was maintained. Following this, salinity was returned to the control value (in control tanks), or reset at the next incremental level (i.e. 2 ppt higher than the previous day). Before the start of each trial, ammonia concentrations were tested in a sample of tanks to ensure bio-filter performance.

3.1.3 Statistical analysis

Survival data from direct transfer and gradual acclimation trials were analysed using Probit analysis (SPSS Version 16.0) This analysis determined the lethal concentration for 10% (LC_{10}), 50% (LC_{50}) and 90% (LC_{90}) of individuals for each trial. For direct transfer trials, LC values were calculated at twelve separate time periods - 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours from the start of each trial. Gradual acclimation LC salinities were calculated once only for each replicate after mortalities of 10%, 50% and 90% occurred. Final species LC values were calculated from the mean of the three replicate trial values.

3.2 Results

3.2.1 Direct transfer toxicity tests

Survival in the control tanks was 100% in the direct transfer trials, (Figure 1), indicating that general laboratory conditions did not adversely affect fish survival and that mortalities in treatment tanks can reasonably be attributed to a treatment effect. The highest salinity treatment (35ppt) had 100% within 12h. The next highest (21ppt) had 10% mortality, within the first 24h. Behavioural observations of speckled livebearer in 35 ppt treatments were that the fish immediately swam up and occupied the surface water layer, whilst in other treatments they mostly remained near the bottom or were evenly dispersed. All other salinity treatments, from 0-12.6 ppt exhibited zero mortality during the experimental period (max 168 hrs).

Probit analysis estimates show speckled livebearer LC values. Estimates of LC_{10}, LC_{50} and LC_{90}, were made for all times (Figure 2). LC estimates decreased after the first 12 hours prior to stabilizing between 12-168h. The species LC_{10}, LC_{50} and LC_{90} values were therefore taken at 168h - the maximum time the trial ran for (Table 1).

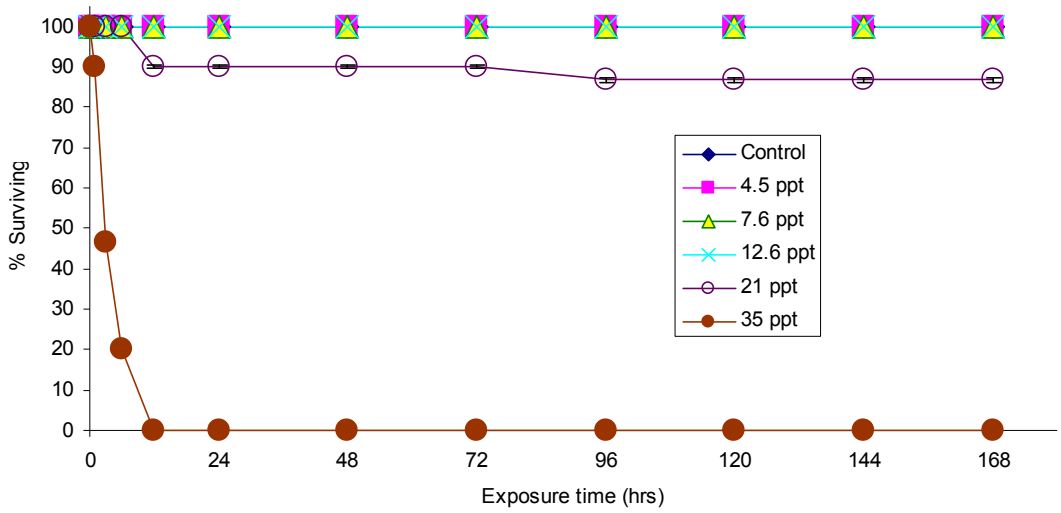


Figure 1: Percent Survival of Speckled Livebearer (mean ± s.e. (*n*=30) in treatments 0-35ppt, for the direct transfer trial over 168h.

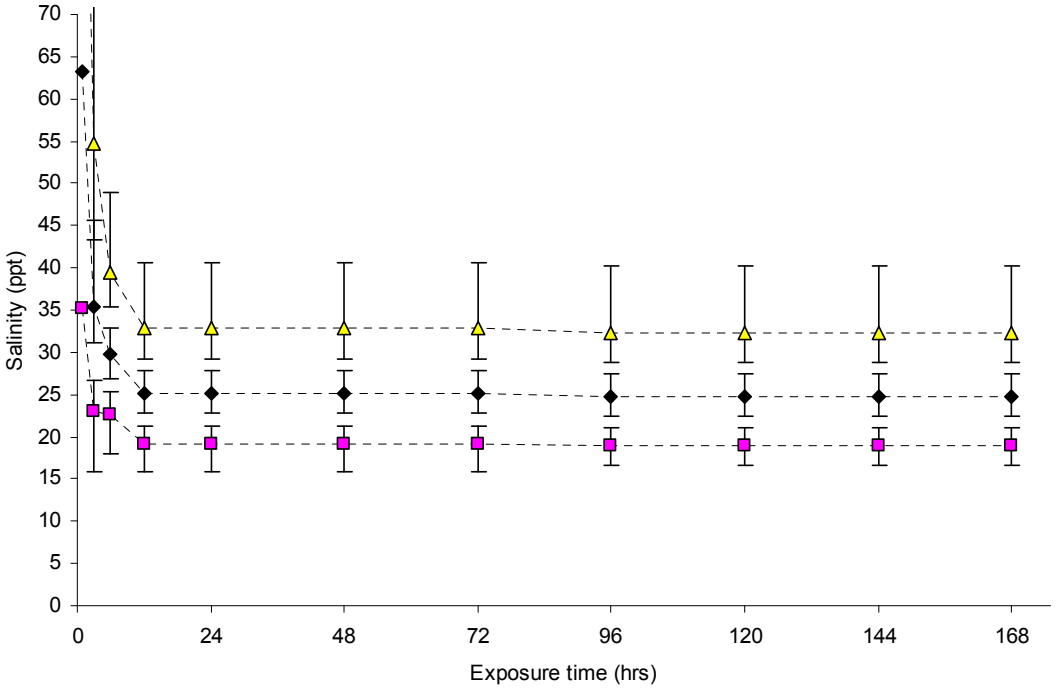


Figure 2: LC_{10} (□), LC_{50} (♦) and LC_{90} (Δ) salinities (± 95% confidence interval (*n*=30) with time, derived from the direct transfer trial for speckled livebearer (n=30).

Table 1: Lethal salinity concentration estimates (ppt from direct transfer ± 95% confidence interval (*n*=30) for speckled livebearer.

LC_{10}	LC_{50}	LC_{90}
18.8 ± 2.2	24.7 ± 2.7	32.3 ± 3.5

3.2.2 Slow acclimation toxicity tests

Slow acclimation trials progressed with no mortalities up to a salinity concentration of 32ppt at which point mortality of treatment fish rose above 10% (Figure 3). Some mortality occurred in the control group, however, this was a small proportion (6.6%) and limited to one tank occurring after day 36. Control mortalities were taken into account during Probit analysis.

Over 90% mortality occurred in salinity treatments by 40ppt and trials ceased at 44ppt following 100% mortality. Slow acclimation LC_{50} estimate are displayed below (Table 2). Behavioral responses were first noted for speckled livebearer at 32ppt when feeding activity was impacted and by 38ppt, hiding, pigmentation, *GVR* and movement were also impacted. Feeding behavior was never severely impacted. Loss of equilibrium was observed by 40ppt with *GVR* also severely impacted.

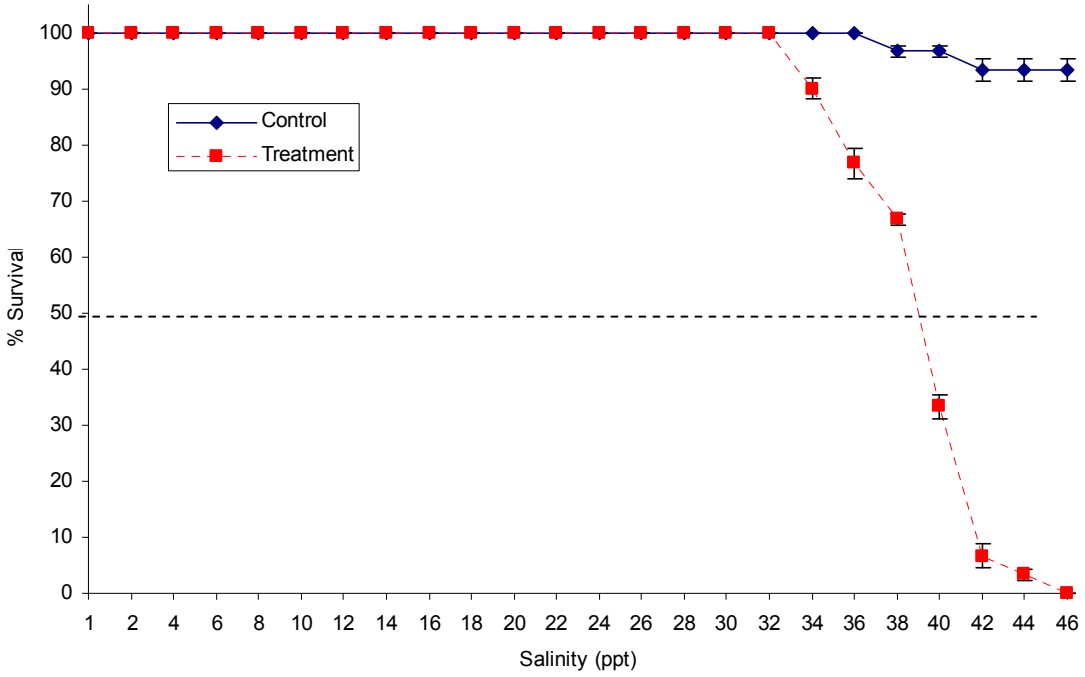


Figure 3: Percent survival for Speckled livebearer (*n*=30) under increasing experimental salinity concentrations (2ppt/day). Error bars indicate standard error around the mean.

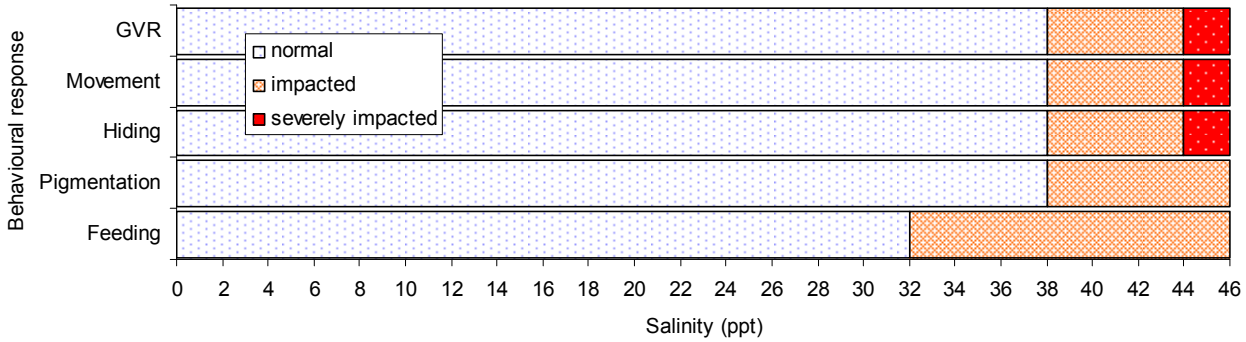


Figure 4: Speckled livebearer behavioural observations with salinity, from gradual acclimation trial (*n*=30).

Table 2: Lethal salinity concentration estimates (ppt from gradual acclimation ± 95% confidence interval (*n*=30)) for speckled livebearer

LC_{10}	LC_{50}	LC_{90}
34.3 ± 1.3	38.4 ± 0.7	42.5 ± 1.3

3.3 Discussion

Estimates from both direct transfer and gradual acclimation methods revealed that speckled livebearer are tolerant of salinities up to approximately that of sea water. This degree of tolerance is exceptionally high for freshwater fishes and indicates that the use of salt as a control method is not feasible. The possibility of using salt was raised by the project steering committee as feasible only if speckled livebearer proved to be exceptionally intolerant of very low levels of salt. These results indicate that the levels of salt required to eradicate the target species would have disastrous consequences on the receiving catchment. Clearly, other control agents such as ichthyocides are likely to be far more desirable for use as field control agents.

The results also indicate that spread of this pest species may not be restricted by estuarine or marine conditions, and they will be highly tolerant of the harsh saline conditions present across much of Australia's inland waterways. Such high tolerances are often characteristics that support widespread invasion and the establishment of introduced species (Moyle and Light 1996). This adds further to concerns that speckled livebearer may become widely established across inland Australia if not rapidly contained and eradicated once populations are discovered in the wild.

4 COMPETITIVE INTERACTIONS WITH MOUNTAIN GALAXIAS

4.1 Materials and methods

4.1.1 Short-term interaction trials – adult Galaxias and livebearers

For short-term competitive interaction trials between Galaxias and Livebearers, 18L experimental aquaria were used with water temperature maintained at 18°C. A small foam filter was supplied to each tank to maintain oxygen mixing, prevent stratification and filter the water. Photoperiod was controlled at approximately 12:12 light dark ratio. Water quality parameters (dissolved oxygen, total dissolved solids, pH and temperature) were monitored daily and ammonia and nitrite were also measured at the start and completion of trials.

Six separate aquaria were used, three aquaria each containing 2 adult mountain galaxias (size range 60-80mm) and 5 adult speckled livebearer (size range 35-50mm) and three aquaria each containing 2 juvenile mountain galaxias (size range 30-40mm) and 5 adult speckled livebearer (size range 35-50mm). This represents a similar methodology to competition trials developed for closely related species (Barrier and Hicks 1994; Rowe et al. 2007). Fish were divided with a partition separating the two species for the first 24 hours to allow acclimation to the tank environment without interaction.

To begin the trials, the divider was removed from each aquarium. Each tank was observed for a ten minute period for any agonistic interactions. Behavioural movements of an agonistic nature were categorised as per Barrier and Hicks (1994), as *intentional movements* towards the other species, *chases*, *contact nips* and *predation* events. The number of observations for each behaviour and notes on competitive behaviours were recorded after 1, 4 and 8 h, then daily for seven days at 24, 48, 72, 96 and 120h from trial initiation. Mortalities and observations of condition were recorded daily and at completion of the trial period. Condition and behavior was assessed subjectively noting obvious indications of stress including discoloration, loss of appetite and erratic swimming behavior.

4.1.2 Long term interaction trials - juvenile Galaxias and livebearers

For long term juvenile Galaxias trials, ten juvenile mountain Galaxias (size range 30-50mm) and fifty adult speckled livebearers (size range 20-50mm) were introduced into each of three 300L outdoor tanks. The trials ran for 62 days and were conducted with flow through bore water at a relatively stable temperature of approximately 17-26°C and natural lighting. Experimental fish were

maintained on a diet of commercially available aquaculture pellets by Skretting®. Weekly measurements of water quality (dissolved oxygen, total dissolved solids, pH and temperature) were recorded. The presence of any disease or health conditions was noted daily, as well as any behavioral changes.

4.2 Results

Whilst no agonistic behaviour was exhibited by the introduced speckled livebearer in lab trials, a number of agonistic and predatory attempts from mountain Galaxias towards speckled livebearers were observed (Table 3). Most agonistic behaviour was exhibited by larger Galaxias (60-80mm), and after 24 hrs 60% of speckled livebearers exposed to large mountain galaxias were consumed; mostly during the night (5pm-8am), although after this point no further mortalities were observed (

Figure 5). Livebearers that survived tended to be larger individuals than those killed.

Table 3: Number of agonistic interactions observed during trial. Results from three replicates pooled. Note: the one contact nip observed was also a successful predation event.

	livebearer aggression towards Galaxias			**Galaxias aggression towards livebearer**		
	Intention	**Chase**	**Contact nip**	**Intention**	**Chase**	**Contact nip**
Adult Galaxias	-	-	-	20	5	1
Juvenile Galaxias	-	-	-	5	3	-

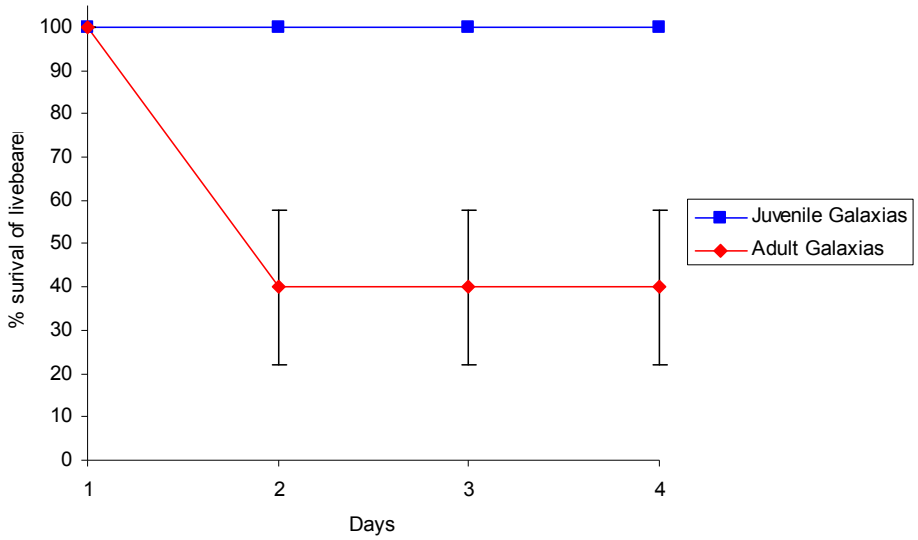


Figure 5: % survival (mean ± SE) of Livebearers during Galaxias competition trials.

It was noted in the longer term outdoor trials that juvenile galaxiids were not large enough to predate on newly born speckled livebearer and were observed to lose condition, growing visibly thinner over the course of the trial. Conversely, in trials containing adult mountain Galaxias, where fish were large enough to consume smaller speckled livebearers, galaxiids were observed to maintain condition during the trial. During the trial period Dissolved oxygen was 7.9 ± 0.3 ppm, total dissolved solids was 1.1 ppt, pH was 7.9 and temperature was 17.8 ± 0.1°C, ammonia and nitrite were below detectable concentrations.

4.3 Competitive interactions with Mountain Galaxias

The lack of agonistic behaviour exhibited by speckled livebearer in the current study concurs with similar results by Maddern *et al.* (2005). The densities used in the current trials were higher than those used by Maddern *et al*. (2005), yet still did not result in interspecies aggression from speckled livebearer. The results suggest that mountain galaxias may predate at low levels on newly born speckled livebearer, although this level of predation may offer little in the way of control.

The composition of dietary items has been investigated for both mountain galaxias (Cadwallader *et al.* 1980) and speckled livebearer (Maddern *et al.* 2005), with considerable overlap between the two species. It is, therefore, likely that speckled livebearers may compete for food resources with mountain galaxias. Additionally, speckled livebearer may consume algal matter that some galaxiid prey items require thereby modifying food-web structure which may impact negatively on galaxiid sustainability. Furthermore, where resources are low it appears speckled livebearer may be able to persist on very low value food resources (e.g. detritus) whilst galaxias can not.

5 PREDATORY INTERACTIONS WITH CONGOLLI

5.1 Materials and methods

Laboratory interaction trials were conducted on adult speckled livebearer (size range 30-50mm) and adult congolli (*Pseudaphritis urvilii*) (size range 150-250mm). A single adult congolli was introduced into each of the three 300L tanks (referred to as congolli 1, 2 and 3) and acclimated to tank conditions over the course of a week. Speckled livebearers were introduced into the tanks on a daily basis. Initially six speckled livebearer were added to the tank. The number of prey fish consumed was recorded each day with the number of speckled livebearer returned to 6 per tank. Weekly measurements of water quality (dissolved oxygen, total dissolved solids, pH and temperature) were recorded. The presence of any disease or health conditions was also noted, as well as any behavioural changes. The trial was conducted over a 30 day period.

Tanks were maintained with re-circulating water at a relatively stable temperature of approximately 18-23°C and 12:12 photoperiod and filtered with a 7500L/hr internal pump linked to an external biofilter. Speckled livebearers were maintained on a diet of commercially available aquaculture pellets, while congolli were fed live earthworms prior to the trial.

5.2 Results

During the first six days, congolli consumed large numbers of livebearers with up to five of the six livebearers consumed daily and with livebearers eaten in all replicates (Figure 4). After six days however, the predation rate dropped considerably with only the occasional single livebearer taken each day, predominantly by congolli 3. By day 4 congolli 1 had ceased to consume livebearers as had congolli 2 by day 13 (Figure 4). Whilst predation rates on livebearer decreased, additional diet items (worms) introduced on days 15 and 25 were eagerly consumed by all congollis, indicating a preference for this food type over livebearer consumption.

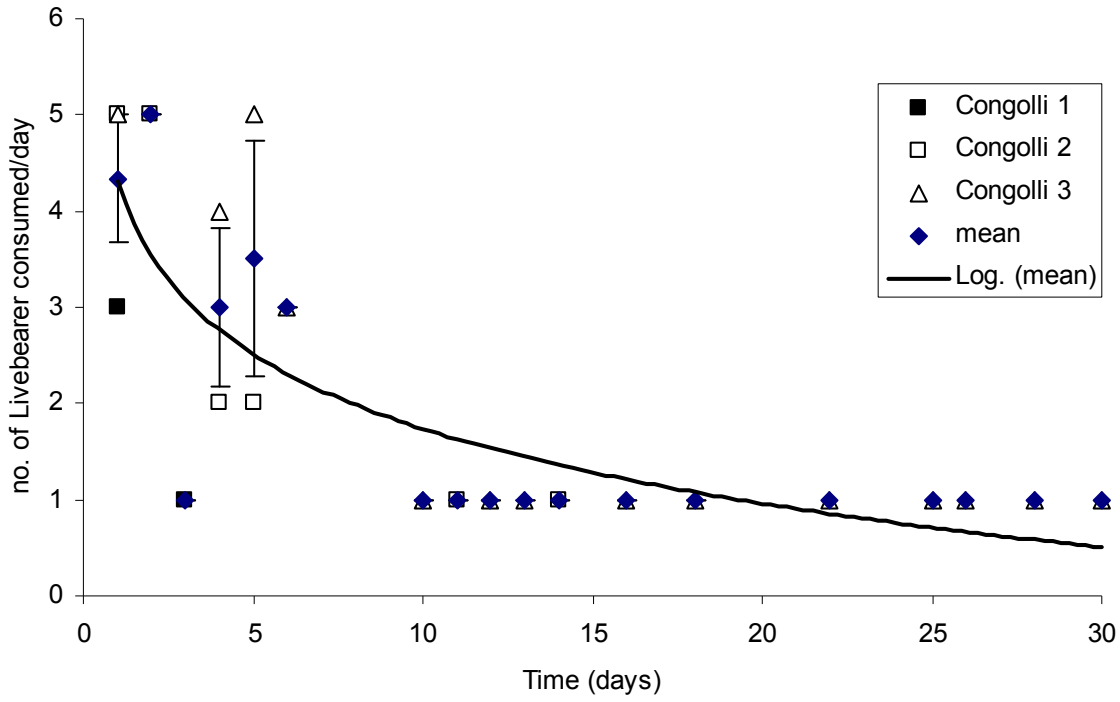


Figure 4: Number of speckled livebearer consumed by congolli per day. Error bars indicate standard error around the mean.

Behavioural observations indicated a predator avoidance response, with livebearers schooling and adhering to edge surfaces within the tank only after the initiation of predatory activity by congolli, before this time livebearers were observed to move freely throughout all areas of the tanks. Livebearers also exhibited avoidance movements, maximising their distance from the congolli at all times, although detailed behavioural data was not collected and is not central to the aims of the current research.

5.3 Discussion

The results show that congolli will consume speckled livebearer under laboratory conditions. However, this consumption drops off fairly sharply after several days and the reasons for this are unclear. Predator avoidance behaviors were observed for speckled livebearers, and these behaviors are likely to contribute to reduced predation success. Alternatively, some livebearers are suggested to possess distasteful chemical composition (Pyke 2008) which may also lead to a 'learned avoidance' on the part of congolli. The readiness of congolli to accept alternative food sources certainly suggests that satiation was not the course of the decrease in prey consumption.

The data suggests that the usefulness of congolli in controlling speckled livebearers in the wild (such as Willunga Creek) is likely to be limited, particularly if alternative food sources are available. In an open and complex stream habitat it would be less likely for congolli to control speckled livebearer due to predator avoidance issues (Eklov and Person 1996; Snickars *et al.* 2004), and predator diet shifts with prey density (Smith and Remington 1996; McEvoy 1996). Furthermore, congolli are catadromous and translocations into Willunga Ck for the purposes of biological control of livebearers would require continual restocking every few years as they are unable to maintain freshwater populations without connectivity to the sea (McDowell 1996, McNeil & Hammer 2007). However, the re-introduction of congolli into Willunga Creek may serve as an effective part of a broader strategy to exert ecological pressure against the livebearer population and may also be a desirable component of a broader ecological restoration program following the successful eradication of livebearers from the creek, but only if the fish passage issue is addressed by reinstating connectivity and access to the sea.

6 ROTENONE AS A CONTROL MEASURE – LABORATORY TRIAL

6.1 Liquid rotenone Pilot Trial

6.1.1 Material and Methods

A series of laboratory trials were conducted to investigate the toxicity of commercial rotenone on speckled live bearers. These trials utilised the direct transfer method outlined previously for salinity tolerance trials. An initial trial was conducted using liquid rotenone under a series of six treatment concentrations (0 (control), 0.05, 0.15, 0.45, 0.135 and 0.405 ppm). These concentrations represent a range spanning concentrations authorised under APVMA permit 9117 (up to a maximum of 0.25ppm). These dosage levels approximate those known to kill another invasive poeciliid species, *Gambusia affinis* (Ling and Willis 2000). Liquid rotenone was supplied by Kendon Chemicals Pty Ltd and was described as containing 9% w/v active ingredient.

The experimental gradient was replicated three times resulting in a total of eighteen separate aquaria. Ten individual fish were randomly assigned to each aquarium and rotenone placed directly into the test tanks, with doses calculated to achieve the predetermined gradient of final concentrations. After the addition of rotenone, the number of mortalities within each aquarium was recorded at ten minute intervals for the first hour. Subsequent to this, mortalities were recorded half hourly until 420 minutes. Observations of condition, including behaviour of the fish, were also recorded and assessed subjectively noting obvious indications of stress including discolouration, loss of appetite and disrupted swimming behaviour.

Experimental aquaria tanks of 18L were aerated and maintained at 21°C. Water quality parameters were measured at the beginning and completion of the trial including: dissolved oxygen, total dissolved solids (as a measure of salinity), pH and temperature. At the start of the trial general (GH) and carbonate (KH) hardness, ammonia, nitrite and nitrate were also measured as it has been suggested these may effect toxicity of rotenone. Water from Willunga Creek was used to ensure that laboratory trials closely matched conditions in the proposed treatment area.

6.1.2 Results & Discussion

All liquid rotenone treatment levels between 0 and 0.405 ppm failed to cause any livebearer mortalities. These results may be attributable to a high tolerance of speckled livebearer to the control agent; however, no data is available on the specific toxicity of rotenone to this species. A pilot trial was subsequently conducted using estimated rotenone concentrations of up to 4.8ppm.

These concentrations proved ineffective for livebearer control raising doubts regarding the quality of the liquid rotenone supplied for the trials. Liquid rotenone suppled may have been defective or deteriorated in storage, mixing or in transit. Subsequent testing of the breakdown rate of rotenone in water suggests that the active chemical may deteriorate rapidly within 24 hours of mixing. There were also difficulties in verifying the actual concentration of active ingredient in the liquid rotenone from the supplier. As a result, the experiment was re-run using fresh powdered rotenone, sourced from an alternative supplier under a different spectrum of concentrations.

6.2 Comparison of Liquid and Powdered rotenone

Comparative trials between liquid and powdered rotenone supplies were run using identical methods to the liquid rotenone pilot (see 6.1) with the exception that the experimental treatment concentrations were 0, 0.25, 0.8, 1.6, 2.4, 3.2, 4.0 and 4.8ppm. Control (0 ppm) treatments received the detergent agents used for rotenone treatments without the addition of any rotenone. Powdered rotenone (9.6% w/w active ingredient) was supplied by Quentin Elvy & Associates Pty Ltd Australia. Powder was mixed with water and a small amount of detergent added.

6.2.1 Statistical Analysis

Survival data from rotenone toxicity trials were analysed using Probit analysis (SPSS Version 16.0) to determine the concentrations lethal to 10% (LC_{10}) 50% (LC_{50}) and 90% (LC_{90}) of experimental fish at a given time.

6.2.2 Results

Survival rate was 100% in the 'detergent only' control treatments (Figure 5 A & B). For liquid rotenone the two highest treatment dosages (2.4ppm and 2ppm) resulted in 100% mortality within 5 hours. At a concentration of 1.6 ppm 100% mortality was observed at the completion of experiment at 7 hours. All other liquid rotenone treatments, from 0.4-1.2 ppt resulted in less than 100% mortality during the experimental period (max 7 hrs), with mortality levels higher under higher dosage treatments.

For powdered rotenone, treatments of 1.2ppm and above resulted in 100% mortality within 50 minutes. At a concentration of 0.8 ppm, 100% mortality was observed at three hours. The 0.4ppm powdered rotenone treatment failed to cause 100% mortality by the completion of the trial at 7hrs, with 10% of livebearers surviving. The 0.25 ppm dosage (the concentration recommended under the APVMA permit) resulted in around 60% mortality after 18 hours exposure. All surviving fish recovered completely and returned to normal levels of feeding and activity.

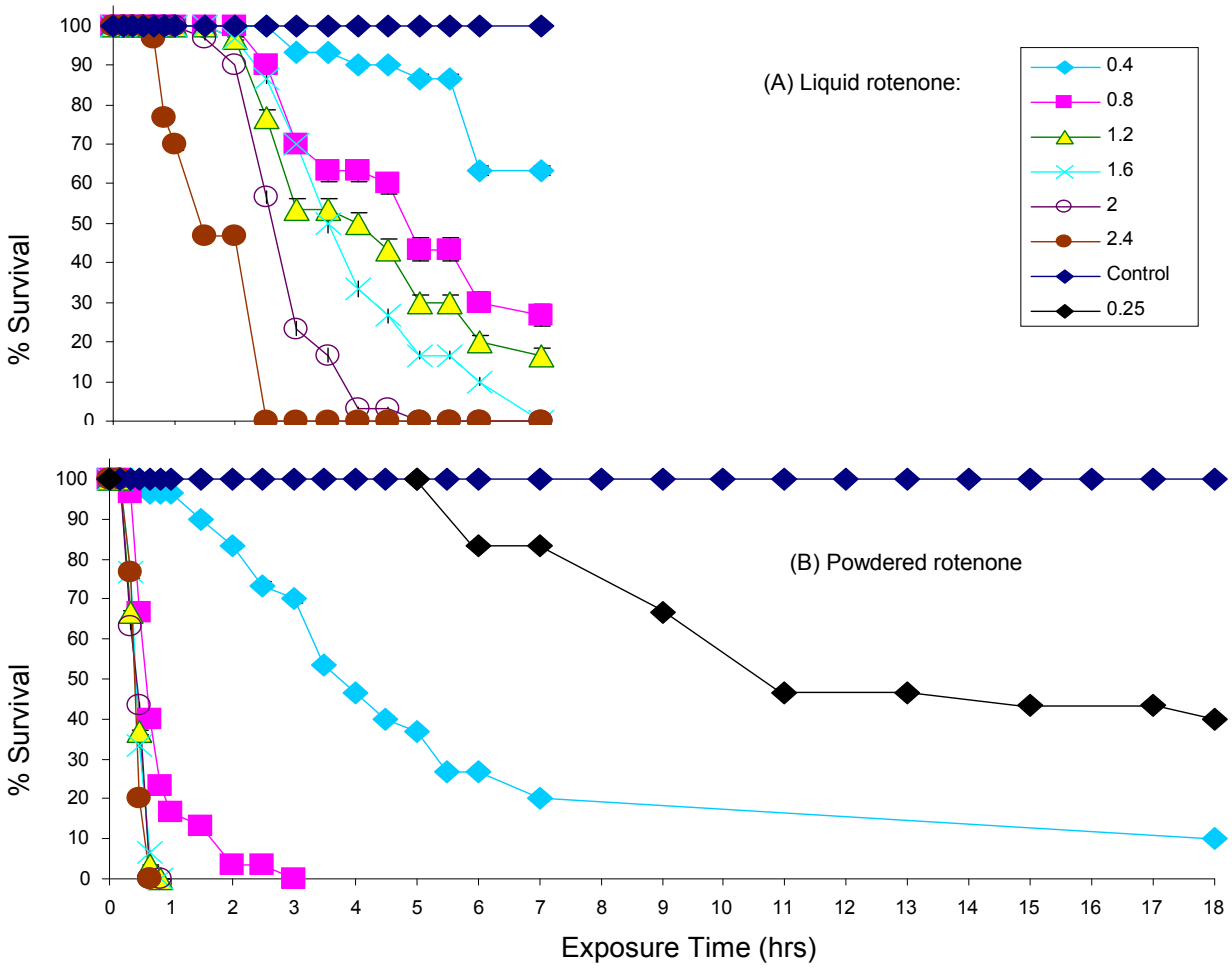


Figure 5: % Survival time of speckled livebearer (*n*=30) in treatments 0-2.4 ppm of rotenone over 18 hours using A: liquid and B: powdered forms from different suppliers.

6.2.3 Lethal Concentration Estimates

Estimates of LC_{10}, LC_{50} and LC_{90}, were made for each of the trial times both for liquid (Figure 6) and powdered (Figure 7) rotenone treatments. LC estimates were generally higher at short timeframes, decreasing and becoming less variable over time. Powdered rotenone trials resulted in far more stable and consistent LC values than for liquid rotenone. The species LC values were therefore taken from the longest time interval possible (7h). The seven hour LC_{10}, LC_{50} and LC_{90} values are listed in Table 4. Liquid rotenone produced LC estimates well above those of powdered rotenone estimating an LC_{50} of 0.63 ppm compared to 0.30 ppm for powder. Behavioral observations show that upon addition of rotenone into experimental tanks, the speckled livebearer initially struggle to maintain equilibrium and often would slowly rise headfirst to occupy the surface water layer, whilst controls mostly remained near the bottom or evenly dispersed.

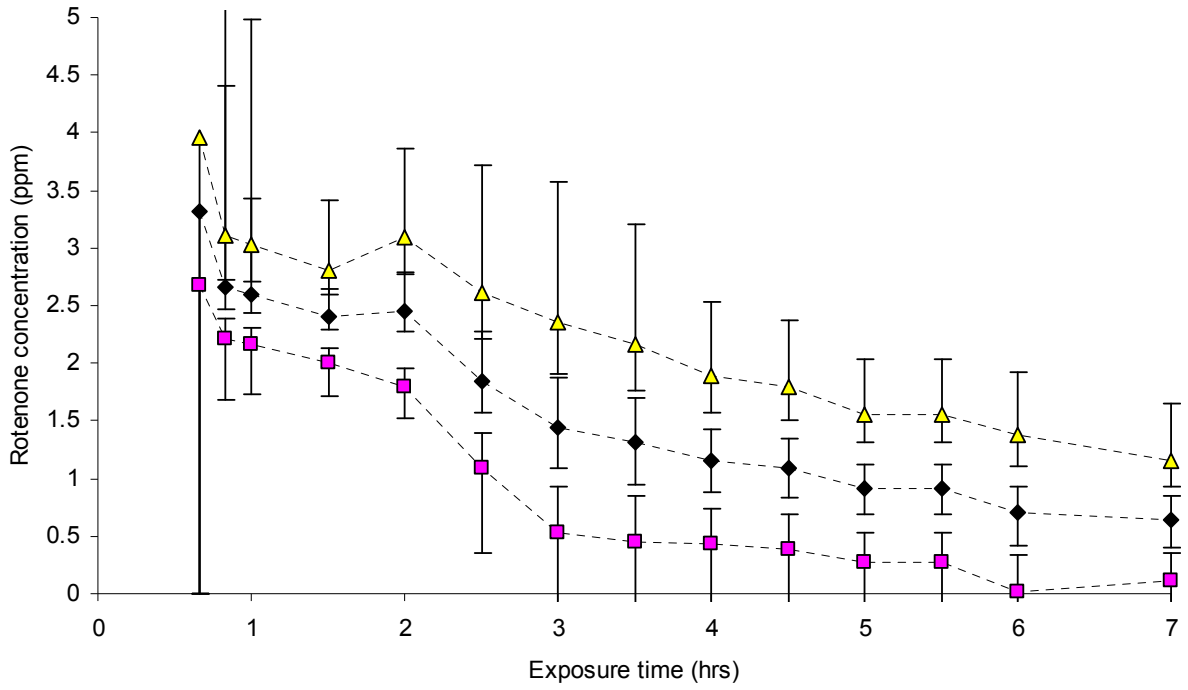


Figure 6: LC_{10} (□), LC_{50} (♦) and LC_{90} (Δ) estimates with time, derived from rotenone trial using liquid rotenone for speckled livebearer (*n*=30). Error bars show upper and lower 95% confidence intervals.

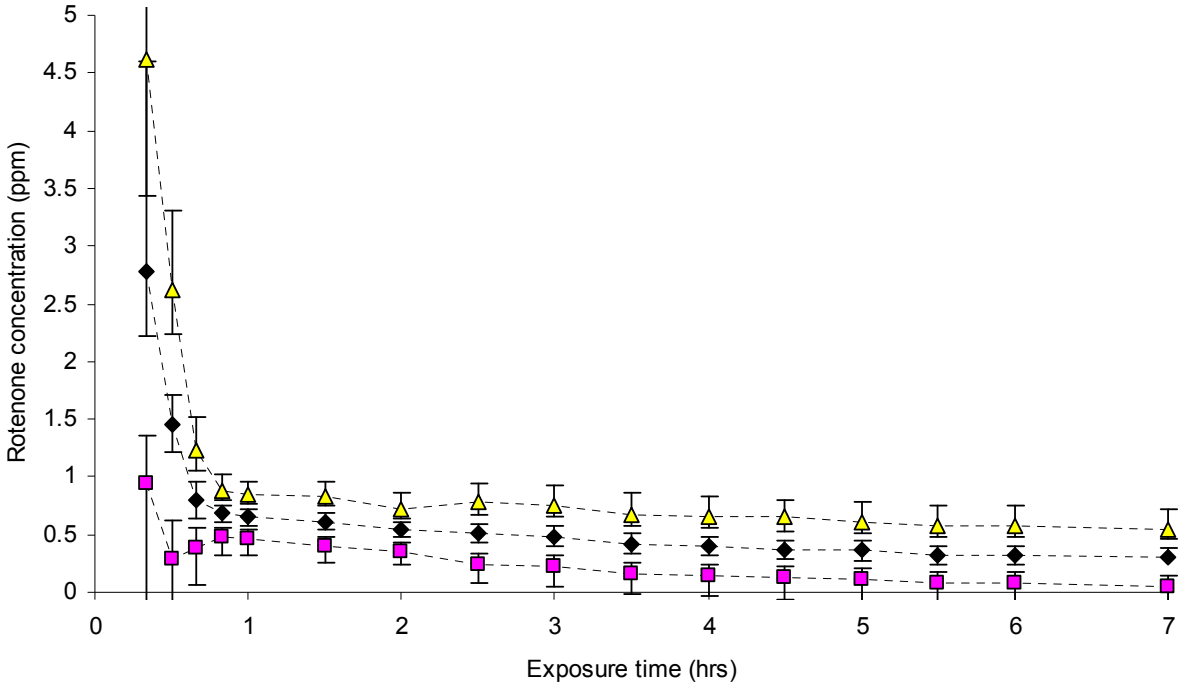


Figure 7: LC_{10} (□), LC_{50} (♦) and LC_{90} (Δ) estimates with time, derived from rotenone trial using powdered rotenone for speckled livebearer (*n*=30). Error bars show upper and lower 95% confidence intervals.

Table 4: Lethal concentration estimates (ppm ± 95% confidence interval (*n*=30) for rotenone toxicity at 7 hours.

	LC_{10}	**LC_{50}**	**LC_{90}**
Liquid rotenone	0.11 ± 0.56	0.63 ± 0.24	1.15 ± 0.23
Powdered rotenone	0.05 ± 0.18	0.30 ± 0.08	0.55 ± 0.17

7 ROTENONE AS A CONTROL MEASURE – FIELD TRIAL

7.1 Field Application Trial

Following the results from the rotenone laboratory trials, a site in Willunga Creek (latitude 35°16'57 S and longitude 138°33'25 E), located upstream of the township of Willunga, south of Adelaide (Figure 8) was selected to undertake a rotenone trial. The trial concentration of rotenone was 0.25ppm in accordance with APVMA permit 9117. The section of Willunga Creek selected for the trial was approximately 51 meters in length and located in the upper reach of the tributary. This section was chosen due to the presence of a large drop off preventing fish moving upstream into this section. An added benefit was that below the site a large water pipe could be sealed off whilst conducting the trial, enabling water to be held back for a number of days until rotenone concentrations had reached below 0.01 ppm in accordance with the permit.

Figure 8: Treatment Site (marked red) for the rotenone application trial near the township of Willunga between St. Johns Terrace and Quarry Rd.

7.1.1 Pre-trial survey

Preceding the rotenone field trial a population survey was undertaken to document the number of speckled livebearer present at the treatment site. To sample the population, two small fyke nets (wing length 3m, mesh size 3mm) and two double winged fyke nets (wing length 5m, mesh size 3mm) were set for a 24 hour period. All speckled livebearer caught were counted and released back into the creek. The flow rate, depth, width and length of the creek and the adjacent pond (Figure 9) were measured to calculate the volume of water and rotenone for treatment of the area.

Figure 9: Main pool at the application trial site with the connected creek channel in the background.

7.1.2 Rotenone trial

The trail was conducted on the 18th December 2009. Before the application began the drainage pipe at the end of the reach was sealed with a specifically designed metal end cap and silicon applied to acquire an adequate seal. For occupational health and safety reasons personal protective equipment (PPE) was worn in the preparation and application of the rotenone, this included: chemical suits, filtered respirators, gloves, glasses and boots (Figure 10). In calculating application rates, the creek was divided into four sections and then the desired amount of powdered rotenone was mixed with water and the surfactant (Polysorbate 80). To apply the rotenone, two 16 litre spray packs and a 120 litre tank with a pump, agitator and extension spray hose were used. The powdered rotenone (9.6% w/w active ingredient) was first mixed with 1 to 2

litres of water and 5 to 10 millilitres of surfactant and then added to the spray pack or tank to then be applied to a section of the creek.

Figure 10: Protective equipment being worn whilst preparing to measure rotenone

The rotenone was applied by spraying in a downstream to upstream direction with each section of the creek being treated separately. To ensure that there was complete mixing, the rotenone was sprayed over the entire surface of the creek and was sprayed from a distance of 1 to 2 meters so that the rotenone could penetrate and mix through the whole of the water column. Careful attention was made to spray in and around any aquatic vegetation. The treatment took approximately 2 hours to complete. Once the application of the rotenone was complete, any speckled livebearers that had lost equilibrium or had died were then collected with a dip net and preserved.

To comply with the APVMA permit 9117, it was required that water testing be carried out to determine the concentration of rotenone in the treated water, indicating when water could be released. Over a 4 day period water samples were taken daily from the trial site and sent for analysis of rotenone concentration. Melbourne SGS Australia Pty Ltd provided analysis and a report on the rotenone concentration. Five days after the trial rotenone concentration was below detectable levels and subsequently water was released from the study site.

7.1.3 Post trial survey

Five days after the water had been released, a follow up survey was undertaken to detect if speckled livebearer were present at the study site. No fish would be able to migrate into this section of the creek due to the barriers present and because the creek was completely dry upstream of the reach. Therefore, any fish sampled must have survived the rotenone application. To sample the population two small fyke nets (wing length 3m, mesh size 3mm) and two double

winged fyke nets (wing length 5m, mesh size 3mm) were set for 24 hours in the same locations as the pre-trial survey. The nets were cleared the following day with all speckled livebearers counted and released.

7.2 Results – Field trial

In the pre-trial survey, 1304 speckled livebearer were captured in the four nets set. At the completion of the rotenone application speckled livebearers were collected using dip nets. It was not possible to recover all fish as some had sunk to the bottom of the creek bed and was unable to be netted. There appeared to be great variation in the reaction of speckled livebearer to the rotenone application with some succumbing quickly and others appearing only slightly affected. Overall, 464 speckled livebearers were removed from the creek over three days from the rotenone application. The post trial survey indicated eradication of speckled livebearer was not achieved with this application of 0.25 ppm. A drop in the numbers of speckled livebearer was apparent, however there were still over 700 fish sampled. Figure 11 depicts numbers of speckled livebearer over two sampling events and mortalities collected as a result of rotenone application.

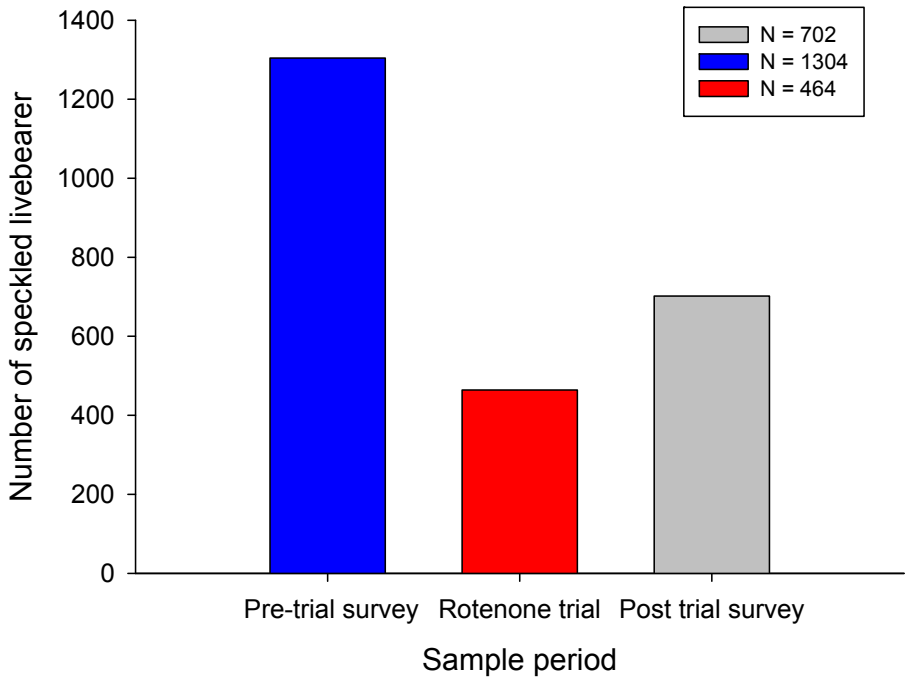


Figure 11: Number of speckled livebearer caught, pre-trial, via rotenone mortality and post-trial.

Comparisons between the pre and post rotenone trial surveys showed a 46% reduction in the abundance of speckled livebearer. This survival rate of 54% is somewhat higher than that observed in laboratory trials (see section 6.0). The results of water analysis for rotenone

concentration by SGS Australia Pty Ltd over the four day period indicated that the intended dosage rate of 0.25 ppm had been delivered. The initial rotenone level was estimated at 0.30 ppm, somewhat higher than the intended dosage of 0.25ppm. The rotenone degraded rapidly within 18 hours of the application and continued to degrade over the following 54 hours. Figure 12 displays the initial rotenone concentration and apparent degradation with time.

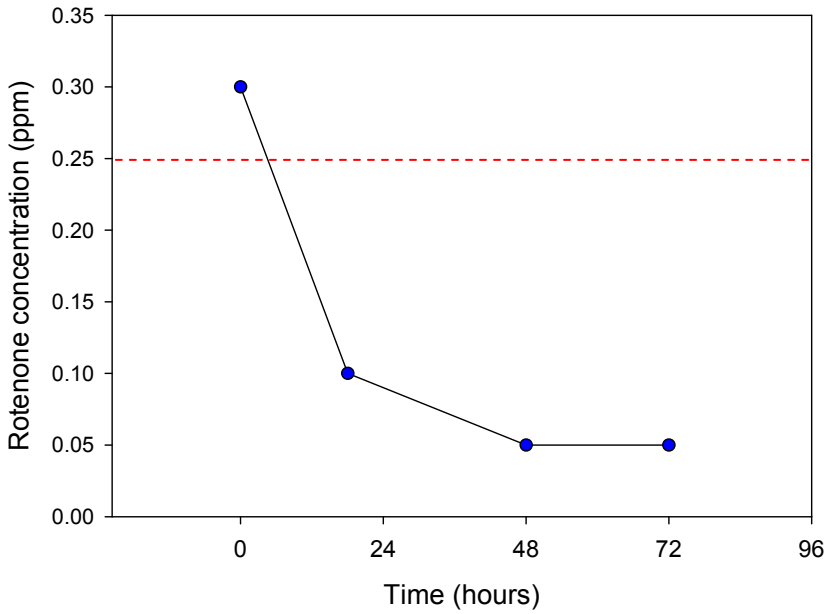


Figure 12: rotenone concentration and degradation with time (over a 72 hour period). Red line indicates APVMA prescribed 0.25ppm for reference.

7.3 Discussion

7.3.1 Rotenone as a control measure.

Rotenone has been widely used to eradicate introduced and unwanted fish species both in Australia (Rayner and Creese 2006) and abroad (Finlayson *et al.* 2000), and laboratory bioassay results from the current study suggest that rotenone will be an effective agent for the control of livebearers in Willunga Creek. However, laboratory and field trial results indicate that the concentration of 0.25 ppm required under APVMA permit 9117, appears insufficient for effective control. In the lab, 40% of speckled livebearer persisted with no apparent ill effects at 0.25 ppm, even after being left in this solution for a long exposure time of 72hours. Indeed, with a 7 hour LC_{50} determined to be 0.3 ppm, and LC_{90} of 0.55 ppm a much higher dosage rate will be required for successful eradication. Rayner and Creese (2006) also found that the species showed a high resistance to the chemical in their eradication attempts, though unfortunately did not publish quantitative results.

The present study suggests that a dosage of at least 1.3 ppm will need to be used to effect complete mortality of livebearers within a short time frame (< 1h) and it is recommended that higher doses be considered to ensure consistent lethal levels throughout the water column and complex habitats present in the field. These results highlight the need for careful species specific testing using reliable rotenone supplies prior to field application trials.

Whilst the field trial dosage of 0.25 ppm was insufficient for eradication, the trial provided an excellent opportunity to test application methods and protocols for a broader control treatment program. The methods for mixing and spraying rotenone were refined with pump spraying the most successful method of application. Backpack sprayers proved useful for smaller areas that may be out of reach of pump spray hoses. It is recommended that large quantities of rotenone be pre-mixed and pumping power maximised to ensure adequate impregnation of the treatment chemical throughout the water column and into dense macrophyte beds. Additional use of trickle feeding may also maximise treatment effectiveness maxing freshwater inflows to ensure treatment dosages are not rapidly deteriorated by inflows from upstream and/or groundwater inputs.

Treatments should be staged in sections and where possible, barriers placed at the downstream extent of the treatment area to prevent upstream colonisation following eradication. Treatment should begin at the upstream extent of the reach to prevent recolonisation from upstream following treatment. Native fauna should, where feasible, be collected and removed from treatment reaches and stored in freshwater tanks off stream. The rapid breakdown of rotenone after 24 hours means that removed fauna can be returned to the reach on the day following treatment, minimising the holding time required. Netting of tadpoles and other aquatic fauna should be carried out during rotenone treatment as most removed individuals recovered quickly once transferred into freshwater tanks.

8 CONCLUSIONS

This series of trials has provided some useful information that will guide the effective and efficient control of livebearers in Willunga Creek. Trials revealed that rotenone applications will be an effective control approach but that dosage rates are required to be much higher than those currently permitted. Permission to use higher dosage rates approaching 2.4 ppm should be sought for the wider control program to be effective. Field application trials refined methodologies for application in preparation for broader treatment of Willunga Creek.

The re-introduction of native fish following livebearer eradication should be pursued, particularly for mountain galaxias, which can be transferred from neighbouring Wirra Creek once eradication occurs. The usefulness of native fish introductions as a control technique, however, was limited

and should not be pursued as a management option. If however, rotenone based eradication proves unsuccessful in the long term, the introduction of galaxiids and congolli may increase the biotic pressure on livebearer populations and improve the natural biodiversity values of Willunga Creek.

It is recommended that a rotenone based control program begin as soon as it is practical during the autumn of 2010 to maximise the chances of eradication prior to winter rainfall and increased creek flows, which may reduce the effectiveness of application methodologies. Whilst there is no evidence that the suggested dosage of rotenone is harmful to non-aquatic species, all applications of rotenone must however, follow approval by the APVMA and should additionally seek approval by relevant legislative authorities to ensure that governance requirements are met. A review of the use of rotenone for pest fish control is available (Rayner and Creese 2006) however it is also suggested that a national approach and consistent protocols for using rotenone also be pursued, preferably in conjunction with the Australian Society for Fish Biology - Pest Fish Committee, which holds significant experience the use of rotenone for the control of freshwater fish.

9 REFERENCES

Barrier, R. F. G., Hicks, B.J. (1994) Behavioural interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxidae) and mosquitofish (*Gambusia affinis* Baird and Girard, 1854). *Ecology of freshwater fish*, 3: 93-99.

Cadwallader P. L. Eden K.A. and Hook, A.R. (1980) Role of Streamside Vegetation as a Food Source for *Galaxias olidus* Günther (Pisces : Galaxiidae) *Australian Journal of Marine and Freshwater Research.* 31: 257-62.

Eklov, P., Person, L., (1996) The response of prey to the risk of predation: proximate cues for refuging juvenile fish *Journal of Animal Behaviour*. 51: 105–115.

Finlayson, B. J., R. A. Schnick, R. L. Cailteux, L. DeMong, W. D. Horton, W. McClay, C. W., Thompson, and G. J. Tichacek. (2000) *rotenone use in fisheries management: administrative and technical guidelines manual.* American Fisheries Society, Bethesda, Maryland.

Green, R. H. (1965) 'Estimation of tolerance over an indefinite time period'. *Ecology:* 46, pp 886.

Koehn, J.D., MacKenzie, R.F. (2004) Priority management actions for alien freshwater fish species in Australia. *New Zealand Journal of Marine and Freshwater Research, Vol. 38*: 457–472.

Ling, N. Willis, K. (2000) Sensitivities of mosqutiofish and black mudfish to a piscicide: could rotenone be used to control mosquitofish in New Zealand wetlands? *New Zealand Journal of Zoology*, Vol. 27: 85-91.

Maddern, M. J., Gill, H. S., Morgan, D. L. (2005) More invasive than *Gambusia holbrooki*? The biology and ecology of the introduced poeciliid *Phalloceros caudimaculatus* in Australia. Supplementary report to the ASFB workshop proceedings 2005.

McDowell, R. (1996) *Freshwater Fishes of South Eastern Australia*, Reed books.

McDowell, R. (2000) *New Zealand Freshwater Fishes*, Reed books.

McEvoy, P.B. (1996) Host Specificity and Biological Pest Control. *BioScience:* Vol. 46, No. 6, pp. 401- 405.

McNeil D. G. Wilson, P. (2008) The speckled livebearer (*Phalloceros caudimaculatus*) A new invasive species for South Australia, SARDI Publication No. F2008/000939-1, SARDI Report Series No. 316. South Australian Research and Development Institute, Aquatic Sciences.

Moyle, P.B. and Light, T. (1996) Fish Invasions in California: do abiotic factors determine success? *Ecology*. Vol. 77: 1666-1670.

Pyke, G.H. (2008) Plague Minnow or Mosquito Fish? A Review of the Biology and Impacts of Introduced Gambusia Species. *Annual Review of Ecology, Evolution and Systematics*. Vol 39: 171-101.

Rayner, T.S., Creese, R.G. (2006) A review of rotenone use for the control of non indigenous fish in Australian fresh waters, and an attempted eradication of the noxious fish, *Phalloceros caudimaculatus. New Zealand Journal of Marine and Freshwater Research*, Vol. 40: 477-486.

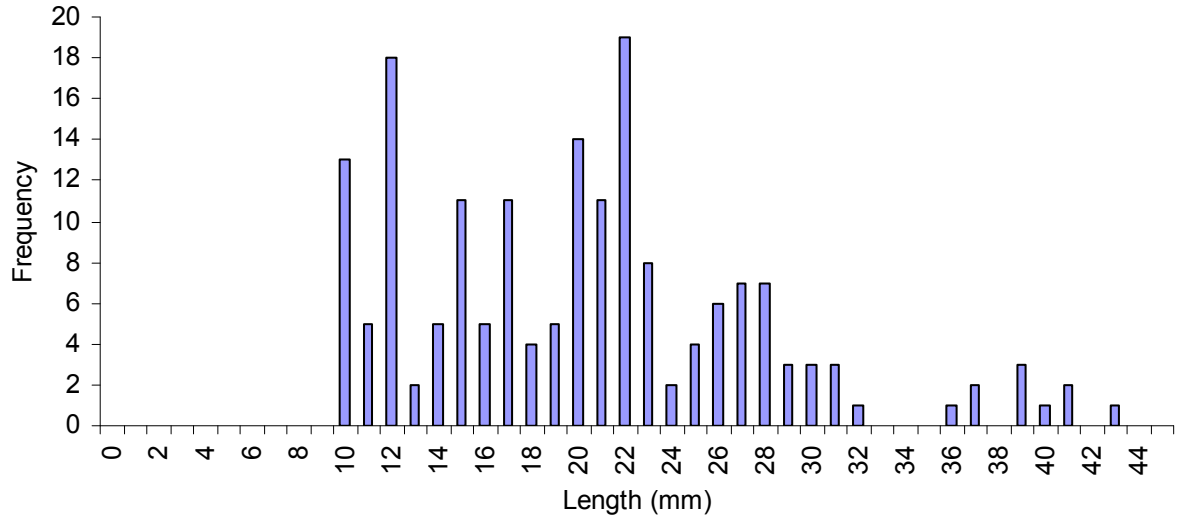
Rowe, D.K, Smith, J. P., Baker, C. (2007) Agonistic interaction between *Gambusia affinis* and *Galaxias maculatus*: implications for whitebait fisheries in New Zealand rivers*. Journal of Applied Ichthyology.* Vol 23: 668-674.

Smith, H.R, Remington C.L. (1996) Food Specificity in Interspecies Competition. *BioScience*, Vol. 46: No. 6, pp. 436- 447.

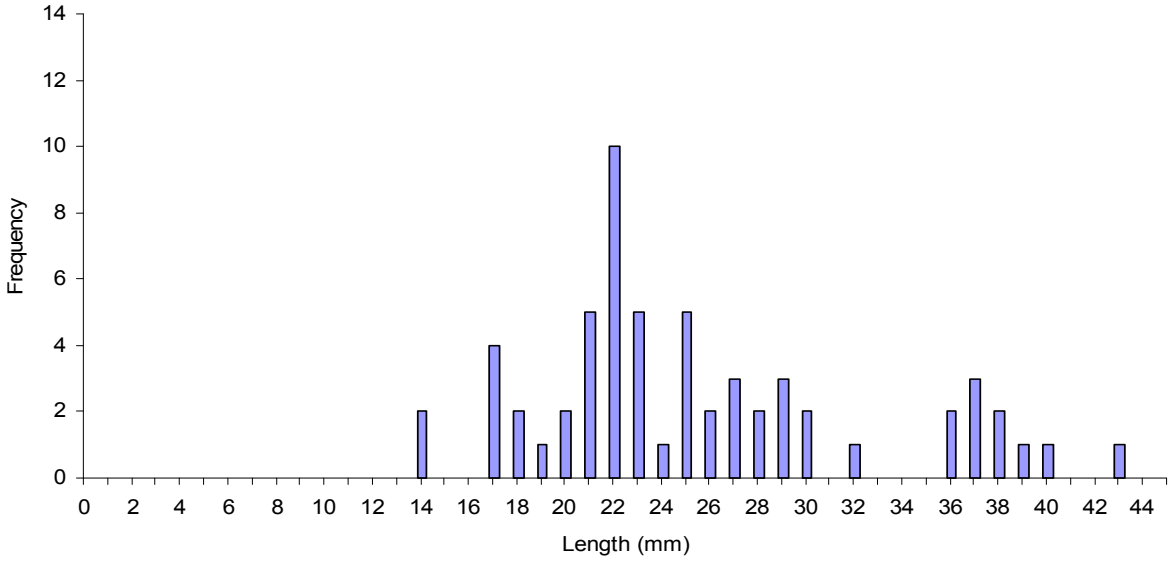
Snickars, M., Sandstrom, A. and Mattila, J. (2004) Antipredator behaviour of 0+ year *Perca fluviatilis* -effect of vegetation density and turbidity. *Journal of Fish Biology* 65, 1604–1613.

Williams, M. D. and Williams, W. D. (1991) Salinity tolerances of four species of fish from the Murray-Darling River system, *Hydrobiologia* 210: 145-160.

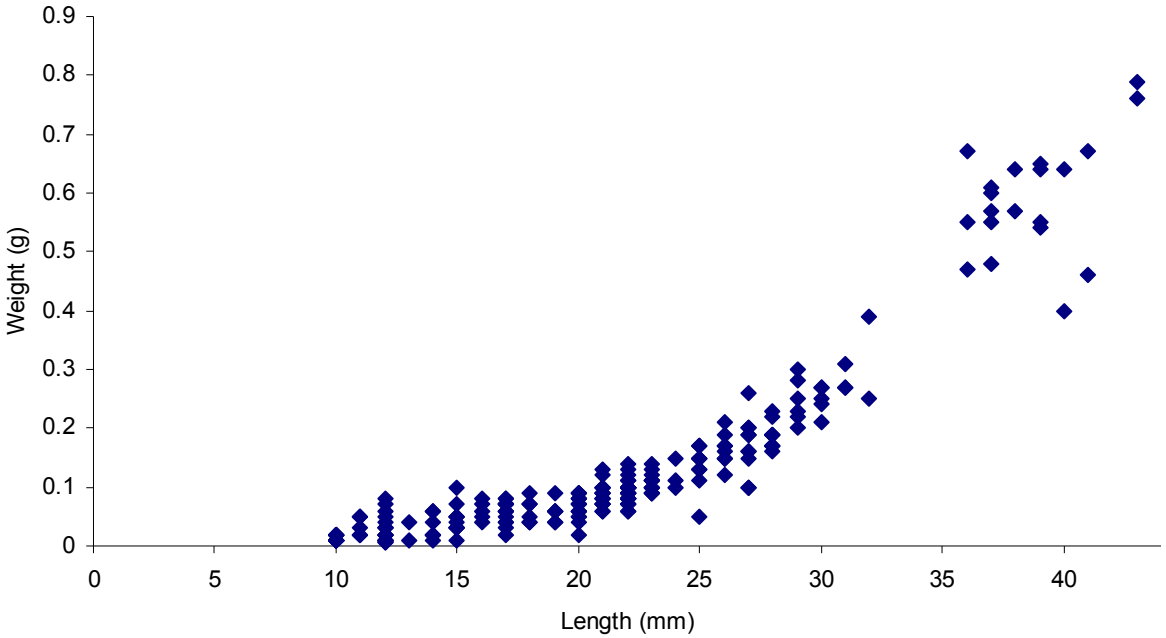
10 APPENDICES



Appendix 1: Length frequency for speckled livebearer (*Phalloceros caudimaculatus*) used in the direct transfer Salinity trial.



Appendix 2: Length frequency for speckled livebearer (*Phalloceros caudimaculatus*) used in the gradual acclimation Salinity trial.



Appendix 3: Length (mm)/Weight (g) relationship for speckled livebearer (*Phalloceros caudimaculatus*) used in direct transfer and gradual acclimation salinity trials combined.