

Improving fingerling production and evaluating inland saline water culture of snapper, *Pagrus auratus*

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ABSTRACT

The major aims of this project were to: (1) identify constraints and develop techniques for management of snapper broodstock to ensure a reliable supply of high quality eggs (2) develop techniques for production of inexpensive, vigorous juvenile snapper, and (3) evaluate the suitability of inland saline groundwater for growout of snapper. Techniques were developed for long-term maintenance of wild-caught and first generation hatchery-reared (G1) snapper broodstock in phototherm controlled, land-based tanks. G1 snapper matured and spawned spontaneously following exposure to ambient and truncated phototherm regimes. The optimum temperature for snapper spawning was identified and high quality eggs can now be obtained all year by maintaining several tanks of snapper with different phototherms. The optimum temperature, salinity and photoperiod (TSP) for intensive, clearwater rearing of snapper larvae was identified in small-scale experiments. When snapper larvae were reared in commercial-scale tanks, growth of larvae in the newly identified TSP regime was 40% faster than that of larvae grown under the original “best-practice” TSP regime. Extensive, fertilised pond rearing of snapper larvae was evaluated at three research and production facilities. Problems were experienced with long-distance transport of larvae and poor initial plankton blooms. In general, survival was low when larvae were stocked between 2-14 dah; however, survival and quality of juvenile snapper were high when snapper larvae were stocked at 20-30 dah into fertilised ponds. Two sources of saline groundwater were evaluated for their suitability for growout of juvenile snapper. These were (1) an evaporation basin at the Wakool/Tullakool Sub-Surface Drainage Scheme (WTSSDS), Wakool, NSW, and (2) groundwater from the Cookes Plains Groundwater Interception Scheme (CPGIS), Meningie, SA. Saline groundwater from the WTSSDS is suitable for culture of snapper in ponds provided the groundwater is fortified with potassium. Snapper grew well during summer and survived low winter temperatures. Acute mortality events occurred on several occasions and were most likely due to pond crashes or severe oxygen depletion due to excessive phytoplankton blooms. Saline groundwater from the CPGIS was also suitable for growth and survival of juvenile snapper. The groundwater did not require ionic adjustment; however, the groundwater is high in iron, which must be removed by aeration and filtration before it can be used to culture snapper. Snapper grew well in a recirculation system housed within a polytunnel (greenhouse), which increased minimum winter water temperatures. There is excellent potential to develop aquaculture of a range of species in saline groundwater at the WTSSDS and CPGIS. However, evaluation of new species and development of pond management practices is necessary in rigorous pilot-scale trials. Expansion of the experimental pond facilities at the WTSSDS is now necessary to conduct replicated experiments to develop techniques for viable snapper culture and evaluate performance of new species.

1. INTRODUCTION

Snapper *Pagrus auratus* is an important recreational and commercial species in Australasia (Bell et al. 1991; Francis 1994). However, there is a substantial short-fall in the supply of snapper to markets in Australia, and catches in New South Wales declined from 1000 t in 1980 to 513 t in 1994/95 (ABARE, 1995). To address this deficit, a total of 684 t of snapper were imported to Australia from New Zealand in 1995. Approximately 98% of this was fresh, whole product which returned an average price of NZ\$6.56/kg FOB (NZ Fishing Industry Board, 1996). A substantial export market for snapper in Asia exists, as evidenced by 2500 t exported from New Zealand to Japan in 1995 (NZ Fishing Industry Board, 1996). The export of live fish from Australia is also developing and the value of the product sold increased from A\$4.6m in 1992/93 to A\$9.0m in 1994/95 (ABARE 1995). A significant, high-value (\$14.50-\$17.00/kg) domestic market for live snapper also exists (Kable, 1996).

In temperate Australian waters, snapper *Pagrus auratus* is seen as an excellent candidate for farming (Battaglione and Bell, 1991). This view is supported by the fact that Australian snapper is the same species as the Japanese red sea bream (Paulin, 1990). The red sea bream has been cultured for more than 30 years in Japan using intensive larval rearing followed by growout in sea-cages (Foscarini 1988). In 1994, production by aquaculture alone totalled 65,000 t. Snapper may also be suitable for stock enhancement, based on successful enhancement programs in Japan (Foscarini 1988).

Pilot-scale snapper production projects have been started in both New Zealand and Australia (NSW, SA and WA) to develop techniques for grow-out of snapper in floating sea cages. NSW Fisheries research has shown that snapper should be suitable for aquaculture because snapper in sea-cages grow at double the rates of wild fish (reaching market size in less than 2 years), readily accept artificial pellets based on agricultural protein rather than fishmeal, and have excellent eating qualities, comparable with wild fish (Bell et al. 1991; Prescott and Bell 1991; Quartararo 1996).

The major bottlenecks to development of commercial snapper aquaculture in Australia are, a reliable supply of good quality eggs and cheap, vigorous fingerlings and access to suitable growout sites are the most important. Hormone-induced spawning of wild-caught snapper usually results in low fertilisation and poor egg quality. Better results have been achieved with hormonally induced captive fish. The aim of this study was to: (1) determine the breeding season of snapper in the wild (2) develop techniques for holding snapper broodstock in sea cages and land-based tanks (3) evaluate the use of hormones to induce spawning in captive broodstock (4) manipulate temperature and photoperiod to induce spawning (5) evaluate various compressed temperature and photoperiod regimes to achieve out of season spawning and (6) evaluate the effect of environmental conditions on sex hormone levels in captive and wild broodstock.

Production of snapper fingerlings has been based upon technology developed in Japan (Battaglione & Talbot, 1992); however, significant problems have occurred during larval rearing in all Australian hatcheries and only relatively small numbers of fingerlings have been produced to date (Battaglione, 1996; K. Frankish, pers. comm., 1997; S. Mawer, pers. comm., 1997; Fielder, unpublished data 1999). Survival of larvae has been unpredictable and highly variable between batches, ranging from 0.8 to 68% (Battaglione, 1996).

Many factors can influence the success of intensive fingerling production and include provision of the appropriate nutritional and physical parameters, and disease control during larval rearing (Blaxter, 1988; Shepherd & Bromage, 1988; Barnabe, 1990; Battaglione, 1996). Several of these factors have already been investigated for snapper production.

The nutritional requirements, in particular n-3 HUFA levels, of Japanese red sea bream are understood well (Kitajima et al., 1980; Watanabe et al., 1983,1989; Izquierdo et al., 1989; Watanabe 1993; Furuita et al., 1996). Techniques and commercial products for enrichment of live feeds, and weaning from live to artificial diets, are also well-developed (Sorgeloos & Leger, 1992). Battaglione et al., (1993) showed that mortality of snapper larvae, particularly at weaning, was significantly reduced when the live food enhancement techniques were used.

Determination of the optimal environmental conditions for larvae and juvenile fish is necessary to maximise production in hatcheries. Three of the most important physical parameters for growth and survival of fish are photoperiod, temperature and salinity (Barnabe, 1990; Battaglione, 1996; Hart et al., 1996). Shepherd and Bromage (1988) state that ideally all factors influencing larval survival should be experimentally tested to find optimal rearing protocols; however, in reality, this level of sophistication is rarely accomplished. Specific rearing protocols have been developed for European sea bass, *Dicentrarchus labrax*, and gilthead sea bream, *Sparus auratus*, the latter of which is closely related to snapper (Barnabe, 1990). Optimal rearing protocols for Australian snapper larvae have not yet been developed.

Because larvae require light to feed, the photoperiod can also affect growth and survival of marine fish larvae. The optimum photoperiod for growth of snapper larvae is unknown; however, visual planktivory of larvae of the closely related gilthead sea bream is optimised when light is provided continuously (Tandler & Helps, 1985; Chatain & Ounais-Guschemann, 1991). Failure for some fish larvae to inflate their swimbladders has been influenced by exposure to light (Battaglione & Talbot, 1990) therefore optimal photoperiod may change with larval ontogeny. The present study investigated the effects of photoperiod on larval ontogeny to determine the optimum photoperiod protocol for growth, swimbladder inflation and survival of snapper larvae. Performance of snapper larvae (as evidenced by presence/absence of food in the gut, swimbladder inflation, somatic growth, and larval survival) was assessed in relation to photic environment.

Water temperature and salinity can directly affect the survival and normal development of fish (Alderdice, 1988; Blaxter, 1988). The biological effects of temperature and salinity are correlated in various ways. For instance, temperature can modify the effects of salinity and widen, narrow or shift the tolerable salinity range; salinity can modify the effects of temperature accordingly (Kinne, 1963). The various developmental states of fish (fertilised egg, larva and juvenile) may have particular temperature and salinity optima (Alderdice, 1988). Tolerance to rapid changes in temperature and salinity may also be influenced by the state of fish development. The present study investigated the effects of temperature and salinity on larval ontogeny to determine the optimum temperature and salinity for larval rearing of snapper fingerlings in intensive indoor hatchery conditions. Performance of snapper larvae (as evidenced by presence/absence of food in the gut, swimbladder inflation, somatic growth, and larval survival) was assessed in relation to physical variables.

Suitable estuarine sites for sea-cage farming in Australia may be limited (Ogburn, 1996); however, potential exists to utilise naturally occurring and man-made inland marine ponds for this purpose. Rising saline groundwater tables and increasing river salinities are major problems in the semi-arid regions of Australia (Simpson & Herczeg, 1994). In order to retain arable land, a method of pumping saline groundwater into purpose-built, on-farm evaporation ponds or sacrificial basins, is currently being used in the wheat-belt of Western Australia, the Goulburn Irrigation area (GIA) of Victoria and the Murray-Darling Drainage Basin (MD) of New South Wales (Otto, 1994; Simpson & Herczeg, 1994; Ingram et al., 1996). Salinity of pumped groundwater varies between sites but ranges from 0-30 ppt (B. Percival, Murray Irrigation Limited, pers. comm., 1997). A range of algae, crustaceans and finfish such as tilapia, red drum, sea bream, eels and channel catfish have been successfully cultured in saline groundwater in North America and the Middle East (Herman,

1991; Rouillard & Robert, 1992; Ingram et al., 1996). In Australia, barramundi have been commercially cultured since 1992, in an indoor, intensive farm in NSW using saline groundwater. Similar methods may be suitable for snapper. The present study assessed the suitability of saline groundwater from the Wakool/Tullakool Sub-Surface Drainage Scheme, NSW and the Cookes Plains Groundwater Interception Scheme, SA for growth and survival of juvenile snapper. Performance of juvenile snapper (as evidenced by somatic growth, survival and food conversion) was assessed in relation to the groundwater chemistry, in particular K^+ concentration.

2. MATERIALS AND METHODS

2.1. Broodstock management

Two 13 000-L tanks were constructed within a photoperiod and temperature (phototherm) controlled room at the Port Stephens Fisheries Centre (PSFC). Each tank was operated by recirculation (~200%/h) with daily 10% exchange of 1 μ m filtered influent seawater. One tank was stocked with 32 hatchery reared first generation (G1) snapper (457 ± 72 g; mean \pm SD) in November, 1994 and the other was stocked with 30 wild-caught snapper (330 ± 119) in April 1995. The fish were fed on rotation daily, pilchards, prawns and squid. Daily food consumption was approximately 3% of body weight.

The phototherm regimes during the project were: Year 1(1995/96), ambient 12 month cycle; Year 2 (1996/97) (Fig. 1) and 3 (1997/98), compressed 4 month cycles (Fig. 2). The phototherm regimes were designed to emulate seawater temperature and daylength at an oceanic reference station situated 3km offshore from Sydney at a depth of 20 m.

Snapper were observed daily for spawning behaviour and spontaneous release of eggs. At the end of each phototherm regime, gonad biopsies were taken from a sample of fish to determine oocyte maturation. In year 3, several fish in both tanks were induced to spawn following implantation of LHRHa cholesterol pellets (~200 ug/kg fish). Eggs from spontaneous and induced spawns were assessed for percent fertilisation and hatching rates.

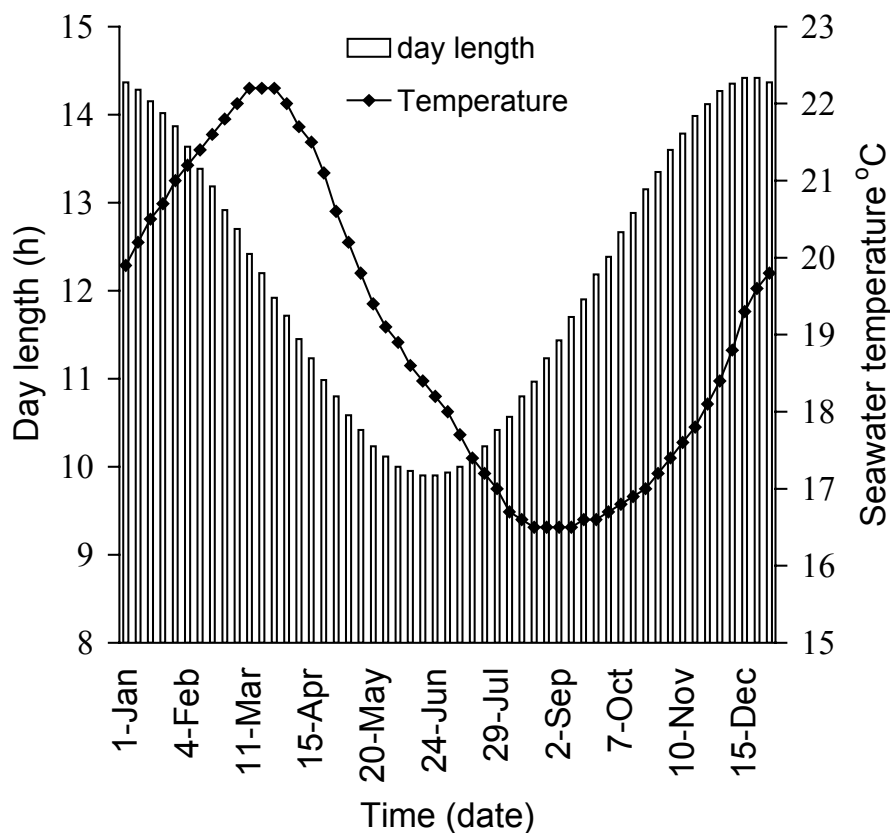


Figure 1. Ambient 365 day temperature and photoperiod regime for snapper broodstock.

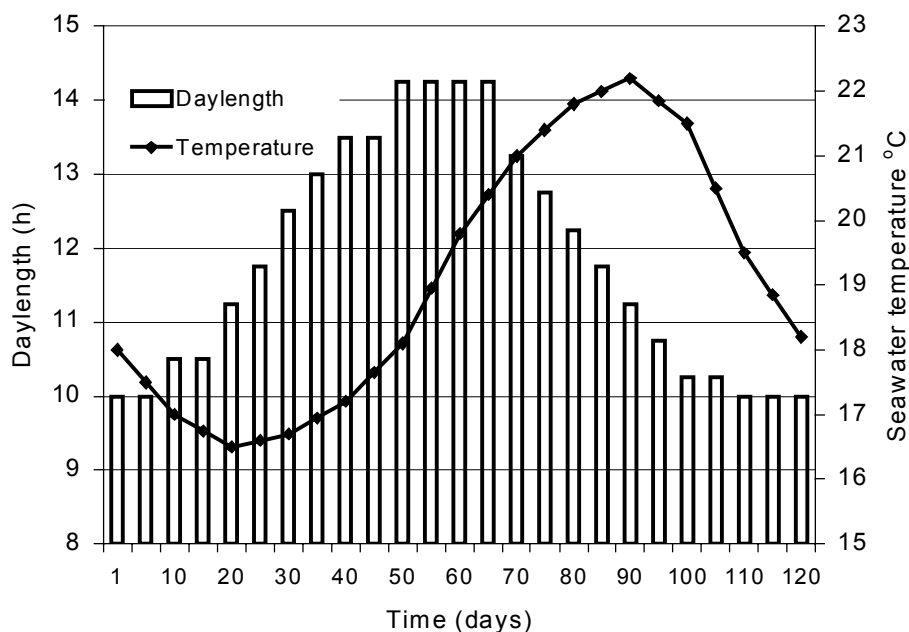


Figure 2. Compressed 120 day temperature and photoperiod regime for snapper broodstock.

2.2. Effect of environmental conditions on sex hormone levels in captive and wild broodstock

Materials and Methods described in Cleary (1997).

2.3. Clearwater intensive larval rearing (PSFC)

2.3.1. General

A series of experiments were conducted in replicated small-scale tanks to identify the optimum photoperiod, temperature and salinity for rearing of larval snapper. All experiments were conducted in 100-l recirculation tanks described by Fielder & Bardsley (1999). In all experiments, 2-3 days after hatch (dah; unless otherwise stated) snapper larvae were stocked at approximately 10-20 larvae/L, and provided with rotifers, *Brachionus plicatilis* at 10/mL. Rotifers were enriched with the microalgae *Pavlova lutheri* and Tahitian *Isochrysis* aff. *galbana*, and DHA Super Selco (Inve, Aquaculture NV, Oeverstraat 7-01200, Baasrode, Belgium) for 24 h before harvest. Snapper larvae (n=10/tank) were sampled every 3 days and measured (total length, TL, mm) and observations made on inflated swimbladders, presence of food in the gut, and presence of urinary calculi. At the end of each experiment each tank was drained and the number of larvae counted to provide an estimate of survival.

2.3.2. Effect of photoperiod on growth and survival of snapper larvae

Two experiments were conducted to determine the effect of photoperiod on growth and survival of snapper larvae.

Experiment 1

This experiment was a range-finding study and investigated the effect of photoperiod (0L:24D, 6L:18D, 12L:12D, 18L:6D, 24L:0D) on growth and survival of snapper larvae (n = 6 tanks/treatment). Water temperature and salinity were maintained at 21°C and 20-35‰, respectively. The experiment was terminated after 12 days (15 dah).

Experiment 2

This experiment investigated the effect of photoperiod (12L:12D, 18L:6D, 24L:0D) on growth and survival of post-swim bladder inflated snapper larvae. Larvae were cultured for 11 days in 2,000-l tanks using techniques described Fielder et al. (Appendix 3), and then stocked into experimental tanks (n = 8 tanks/treatment). Water temperature and salinity were maintained at 21°C and 20-35‰, respectively. The experiment was terminated after 21 days (32 dah).

2.3.3. *Effect of temperature on growth and survival of snapper larvae*

This experiment investigated the effect of water temperature (15, 18, 21, 24, 27, 30, 33°C) on growth and survival of snapper larvae (n = 4 tanks/treatment). Water salinity and photoperiod were 35‰ and 14L:10D, respectively. The experiment was terminated after 18 days (21 dah).

2.3.4. *Effect of salinity on growth and survival of snapper larvae*

This experiment investigated the effect of salinity (5, 10, 15, 20, 25, 30, 35, 45‰) on growth and survival of snapper larvae (n = 3 tanks/treatment). Water temperature and photoperiod were 21°C and 14L:10D, respectively. The experiment was terminated after 18 days (21 dah).

2.3.5. *Effect of interaction of temperature and salinity on growth and survival of snapper larvae*

This experiment investigated the interaction of salinity (20 and 35‰) and temperature (18, 21, and 24°C) on growth and survival of snapper larvae (n = 5 tanks/treatment). Photoperiod was maintained at 14L:10D. The experiment was terminated after 18 days (21 dah).

2.3.6. *Commercial-scale evaluation of optimum photoperiod, temperature and salinity identified in small-scale experiments*

This experiment was designed to evaluate the optimum photoperiod, temperature and salinity regimes identified in small-scale experiments with the original “best-practice” optima (control) used at the beginning of the project, on growth and survival of snapper larvae in 2,000-l tanks described by Fielder and Bardsley (1999) (3 tanks/treatment). The treatment physical regimes were: (a) new; 24°C, 35‰ diluted to 20‰, 12L:12D to swimbladder inflation then 18L:6D and (b) control; 21°C, 35‰, 14L:10D. Approximately 60,000 (30/l) 2 day old larvae were stocked into each tank and fed using a standard regime of rotifers, *Artemia* and “ML powered” weaning diet (Nippai Formula Feed Manufacturing Co., Japan). The experiment was terminated after 30 days (32 dah).

2.4. *Clearwater commercial trials, Pisces Marine Aquaculture (PMA)*

Nine shipments of newly-hatched snapper larvae (range 10,000 to 965,000 larvae; total approximately 2×10^6 larvae) were transported from PSFC to PMA for rearing in a commercial hatchery at Brooms Head, NSW. A range of rearing techniques was used and included: clearwater and greenwater; static tanks with daily drain-down and top-up of exchange water; flow-through

tanks with exchange ranging from 20% to 100% volume/day; tank size ranged from 1,700 to 10,000 L. Feeding regime included rotifers from 3 dah; *Artemia* nauplii from approximately 15 dah, followed by enriched *Artemia* metanauplii; weaning diet (Nippai ML-powered; and Lansy, Artemia Systems, Belgium) was generally offered after metamorphosis (> 30 dah). All rotifers and *Artemia* metanauplii were enriched primarily with Selco products or with the microalgae *Pavlova lutheri* and Tahitian *Isochrysis* aff. *galbana*. The algae was supplied in a concentrated form from PSFC.

2.5. Greenwater larval rearing

Two experiments, one each at PSFC and Bribie Island Aquaculture Research Centre (BIARC), respectively were conducted to evaluate the suitability of the greenwater culture technique described by Palmer et al. (1992) for rearing of snapper larvae.

2.5.1. Experiment 1 (PSFC)

A 30 day greenwater experiment was conducted at PSFC in 3 replicate, 10, 000-L outdoor tanks. Each tank was initially filled with sterilised estuarine seawater, fertilised with 35g Aquasol and inoculated with 15% of total volume of microalgae, *Nannochloropsis oculata*. Snapper larvae (3 dah) were stocked at 10/L into each tank.

The tanks were principally static for the duration of the trial with new algae added as required (range 4 to 19% total volume) to maintain algal densities around 1×10^5 cells/ml. The feeding regime consisted of: rotifers at 5/ml from 3 to 32 dah; artemia from 24 to 32 dah; and Nippai ML-powered pellet, 100 and 250 μ m from 18 to 32 dah. Both rotifers and *Artemia* (> 48 h old) were enriched prior to feeding to larvae with the microalgae *Pavlova lutheri* and Tahitian *Isochrysis* aff. *galbana*, and the commercial DHA Super Selco (Artemia Systems, Belgium).

A handling stress test, based on normal procedures that hatchery-reared fish have to endure, was developed to quantify the quality of the fish. The test involved exposing fish to the air for 30 sec in a dip-net and then transferring them to a beaker of water which had been inoculated with *Artemia* nauplii at 0.1/ml. Fish were then observed immediately, 0.5, 1.0, 3.0 and 24 h after transfer for mortality; fainting (presence/absence); abnormal swimming behaviour; feeding response (presence/absence).

2.5.2. Experiment 2, BIARC

The aim of this study was to compare hatchery-based production of juveniles with a combined hatchery/pond production cycle. Larvae were stocked into ponds at two different ages (day 20 and 30) to assess relative suitability for pond-rearing. The trial also provided some comparative data on greenwater hatchery methods as opposed to clearwater methods used elsewhere.

Approximately 200,000 snapper eggs and newly-hatched larvae were transported from PSFC to BIARC in oxygenated drums. Larvae were divided evenly in number and placed into four 5,000-l larval culture tanks (LRT), each filled to 1,800-l with greenwater (*N. oculata* at approximately 2×10^6 cells/ml). Water temperature and salinity were maintained at 23°C and 33‰, respectively and rotifers were added at 20/ml. Modifications to techniques described by Palmer et al. (1992) included: provision of a surface skimmer in each tank, addition of 50% of the live feed enrichment product prior to completion of swimbladder inflation, and feeding of newly hatched brine shrimp from day 20. Newly hatched brine shrimp were replaced by enriched, on-grown, brine shrimp after two days (enrichment product Sanders Docosa Gold, USA). Water conditions and food densities were monitored daily.

On day 20, two LRTs were harvested and the larvae transferred to two prepared nursery ponds (0.02ha). At this time survival, length and accurate swimbladder inflation rates were determined. Ponds N3 and N4 received 26,500 and 12,200 larvae, respectively. At this time final survival, length, and quality (evaluated by standardised stress test) were determined. Although this completed one half of the trial, these fish were transferred to the hatchery for weaning, and those lacking a swimbladder were separated and discarded.

The two remaining LRTs were maintained in the hatchery to day 30, and to this time were fed on enriched brine shrimp, although small numbers of rotifers were retained. On day 30, these two tanks were harvested, and final survival, length, swimbladder inflation and quality determined. Fish from one of the tanks (7,510) were transferred to a third prepared nursery pond (N1), while fish from the other (16,770) were maintained in the hatchery for weaning. Survival, length and stress test results for juveniles from this pond at harvest were assessed. Again, fish from this harvest were transferred to the hatchery for removal of fish without a functional swimbladder, and weaning.

2.6. Extensive fertilised pond larval rearing

Experiments were conducted at a commercial marine fish hatchery (Glen Searle Aquaculture, Yamba, NSW) and PSFC to evaluate the suitability of extensive fertilised pond rearing technique for culture of snapper larvae.

2.6.1. Glen Searle Aquaculture (GSA)

Four experiments were attempted to rear newly-hatched snapper larvae and advanced fry in earthen ponds at GSA, Yamba, NSW. Pond fertilisation regimes and pond management schedules, which were used in the trials were developed by Glen Searle over many years to culture of a range of marine and freshwater fish larvae. Experimental design ranged from single-pond to replicated-pond trials and was dependent on availability of ponds due to commercial operations.

Experiment 1

This study was initiated in September 1997, to determine the effect of artificial heating (partially-controlled greenhouse environment) on growth and survival of snapper larvae in fertilised ponds. Four, replicate, 250 m² ponds were each filled with brackishwater (25‰), fertilised and stocked with 37,500, 3 dah snapper larvae. Two ponds were housed within a greenhouse, and two (control) ponds were exposed to ambient conditions. Ponds were drained after 10 weeks to estimate survival of snapper larvae.

Experiment 2

This study was initiated in October 1997, when 275,000 excess snapper larvae were available from PSFC. Larvae were transferred from PSFC and stocked into one 0.25 ha fertilised pond. The pond was drained after 4 weeks to estimate snapper larvae survival.

Experiment 3

This study was initiated in April 1998 to determine the effect of age of stocking of snapper larvae (7 and 14 days after hatch) on growth and survival in fertilised ponds. Larvae were reared in 2,000-l intensive clearwater tanks using techniques described by Fielder and Bardsley (1999). Approximately 5000 larvae were stocked into each of three replicate 250 m² ponds per treatment. Ponds were drained and harvested after 6 weeks to estimate survival of larvae.

Experiment 4

This study was initiated to determine the growth and survival of advanced juvenile snapper in a fertilised pond. Approximately 12,000 juvenile snapper (40 dah) were cultured in a 2,000-L clearwater tank at PSFC and then transferred in oxygenated drums to Yamba. Juvenile snapper were divided evenly in number and distributed into each of two, 250m² fertilised ponds. After 46 days, both ponds were drained to estimate survival of snapper.

2.6.2. Extensive pond trials (PSFC)

This study was initiated to investigate the effect of age of snapper larvae on growth and survival when stocked into fertilised ponds at PSFC. Larvae were stocked into 500 m² plastic-lined ponds filled with fertilised seawater at 4, 8 and 16 dah (n = 2 ponds/age). When larvae were 40 dah, the ponds were drained and fish harvested. Age of stocking significantly affected survival of larvae; however, survival was low in all treatments. Larvae stocked at 4 dah had significantly lower survival (0.35 ± 0.49 %) than those stocked at 8 (1.8 ± 0.04 %) and 16 (2.1 ± 1.9 %) dah, which did not differ. Growth of larvae was rapid (range 0.8-1.0 mm/d) and did not differ between treatments.

2.7. Saline groundwater growout

2.7.1. Small-scale laboratory experiments (PSFC)

A series of laboratory bioassays was conducted at PSFC using saline groundwater which was collected from (1) evaporation basins at the Wakool/Tullakool Sub-Surface Drainage Scheme (WTSSDS), Wakool, NSW and transported to PSFC.

The aim of the experiments was to determine the suitability of saline groundwater from the WTSSDS for growout of juvenile snapper. Experimental design and methods are reported in Appendix 3.

2.7.2. Pilot-scale pond experiments (WTSSDS)

A pilot growout trial was initiated at the WTSSDS to determine if snapper could be cultured in inland saline water. Specific aims were to (1) determine if snapper could be cultured in open, static evaporation ponds at Wakool, NSW and (2) compare the quality (external colour, taste) of the snapper grown in inland saline water with wild-caught and seacage cultured snapper.

Trial 1

A 0.15 ha unlined earthen pond was constructed by Murray Irrigation Limited within an existing 30 ha saline groundwater evaporation pond. The pond was built in a corner of an existing pond and therefore used two existing pond walls. The new experimental pond walls were constructed with clay, which had been collected from a site nearby to the ponds. The pond was then drained and allowed to dry for 2 weeks prior to filling with saline groundwater from the adjacent evaporation pond. Potassium, as potash was then added to the groundwater. On 2 December 1998, 600 juvenile snapper (22 g) were transported from PSFC and stocked into the pond. Several hours after stocking many fish were swimming close to the water surface and were not responsive to external stimuli. After 24 h hundreds of dead fish were collected from the pond edges and bottom. No healthy fish were ever sighted after stocking and it was assumed that all fish had died soon after stocking. The rapid cause of death indicated the likely cause of death as a dissolved toxin such as hydrogen sulphide. Prior to the pond trial, 20 juvenile snapper had been held successfully in a

2,000-L tank at Wakool for several months using fortified saline groundwater and an internal biological filter to control dissolved ammonia.

Investigation of the pond sediment showed that an extensive layer of organic sludge covered the pond bottom. During pond construction, it was not possible to remove any sludge, as the mechanical process was viewed as potentially causing problems with the pond bottom and may have resulted in a leaking pond. Samples of sludge and clay from old and new pond walls were transported to PSRC and replicated bioassays were conducted to determine if the sediment was causing death of snapper. Results of the trials showed that death of fish was induced when fish were exposed to sludge. Based on this, it was determined that the best option for the viability of the project was to place a plastic liner within the pond and potentially any extraneous problems caused by pond construction or design. A liner was installed in early May 1999.

The pond was refilled with fortified groundwater, and on 12 May 1999 a total of 240 juvenile snapper (109 g) were stocked into the pond to determine growth and survival during an annual cycle under ambient environmental conditions. In addition, 10 snapper were placed into floating cage $\sim 0.5\text{m}^3$ to allow daily observation of fish health and behaviour. Water pH, Dissolved oxygen, Temperature and salinity were measured daily. Fish were not sampled for the first 153 d post-stocking to avoid stress associated with low water temperature and handling.

The estimated number of fish after the first sample was low and a further stocking of advanced snapper was conducted to determine growth and survival of snapper during the summer. Fish were tagged externally to distinguish between surviving, untagged snapper stocked in May. The aim was to compare the performance of snapper, which had survived winter in the pond and snapper, with those fish that were introduced to the pond after winter.

Trial 2

The pond was drained completely, cleaned and refilled with saline groundwater fortified with potassium. On 8 March, 2000 a total of 96 snapper (mean weight – 241 g) were stocked into the pond and fed as in Trial 1.

Cage trial

A total of 199 snapper (mean 5.2 g) was transferred from PSFC to a single floating cage within the 0.15 ha pond on 12 October 1999. Aims were to determine if (a) a slow (12 h) acclimation protocol from transporting conditions to pond water could alleviate perceived post-stocking mortality and (b) to determine growth and survival data for snapper held in cages in saline groundwater during summer. After 43 d, fish were divided evenly into two identical 1m^3 floating cages. Samples of fish ($\sim n = 50$) were taken every 14 d to provide an estimate of fish weight.

2.8. Polytunnel trials (SARDI, Coorong)

2.8.1. Trial 1

A 10,000-l recirculating tank was installed in a polytunnel greenhouse at the Cookes Plains Groundwater Interception Scheme, Meningie, SA. In October 1997, 3,000 juvenile snapper were transported from a commercial hatchery at Arno Bay, Eyre Peninsula, SA and stocked into the tank. Approximately 1,100 of fish died in the 3 days following stocking. It was assumed transfer stress attributed to inappropriate husbandry prior to transport, was the main cause of mortality.

The tank was then restocked with approximately 1,900 snapper (mean wet weight - 1.6 g) which were grown for 30 day. Fish were fed Gibsons extruded barramundi diet following a commercial

feeding protocol based on water temperature, fish size and estimate of tank biomass from mean weight and mortality records. Daily records were kept for feed consumption, water and air temperature, salinity and ammonia levels within the culture system. Growth checks on 100 fish were conducted every 10 days.

2.8.2. Trial 2

In late 1998 the mechanical and biological filtration components of 2 x 10,000-l tanks were upgraded. One tank was stocked on 16 June with 2,000 juvenile snapper (0.5g) with the aim to on-grow to 400 g. The second tank was stocked on 4 November 1998 with 200 x 395g average snapper purchased from Spencer Gulf Aquaculture (SGA) with the aim to on-grow to 1 kg. The aim was to determine the growth of snapper from 0.5g - 1.0 kg in recirculating tanks and to provide some production data on which to base further research and for investors interested in growing snapper using saline groundwater. Power failure occurred on 8 December resulting in death of all fish.

In January 1999 a further 200 juvenile snapper were collected from Clean Seas Aquaculture (CSA), Arno Bay, Eyre Peninsula, SA. These fish were stocked into a 10,000L recirculating system within a polytunnel. These fish have been cultured on commercial snapper pellets (Eyre Peninsula Aquafeeds) to assess growth performance to market size.

3. RESULTS

3.1. Broodstock management

At the end of July 1995, behaviour of the G1 snapper changed and natural spawning was first observed on 1 August when the water temperature was only 15°C (Fig. 3). Spawning continued intermittently for over a month with peak collections of around 250 000 eggs/d. Fertilisation rates were generally lower than 10%, except for one egg collection with 50% fertilisation. The reasons for poor fertilisation rates were unknown, however the small number of mature male fish may have contributed. The tank temperature was increased to 18 °C, the pH buffered to 8.2. Total Copper concentration was 0.05 mg/l and total Aluminium concentration was 0.22 mg/l. These were both in fact lower than the influent exchange (control) water of 0.10 and 0.39 mg/l respectively. Some fish were hormone induced to spawn and produced viable larvae. The wild-caught fish did not display any change in behaviour and did not spawn. The fish were left undisturbed until the next breeding season in spring 1996.

In 1996, the G1 snapper began spontaneous spawning on 9 July which corresponded to an increasing daylength of 10.5 h (from minimum of 10.0 h) and water temperature of 16.0 °C. Once spawning had commenced, the photoperiod was kept constant at 10.5 h, and only temperature was altered. Temperature had a major effect on the development of eggs. When spawning occurred between 15.5-18.5 °C egg development was generally poor (Fig. 4). Initial fertilisation rates were often high (>70%) however egg development was abnormal. If total mortality did not occur, abnormal egg development included: misshaped embryos; reduced yolk volume i.e. the yolk often shrank away from the chorion; opaque spots throughout the egg. Resulting larvae generally lacked vigour and had small yolk reserves.

When spawning occurred between 19-23°C (temperatures greater than 23°C were not imposed) however, fertilisation rates, egg quality and development, and larvae quality were generally excellent (Fig. 4). Both fertilisation and hatching rates of >90% were common. Although spawning continued to occur at low temperatures (15°C), the frequency of daily spawning was lower than that experienced when temperatures were high. The G1 snapper spawned for almost 5 months from 9/7/96 - 1/12/96. The numbers of eggs spawned ranged from about 50,000 – 800,000 eggs/d.

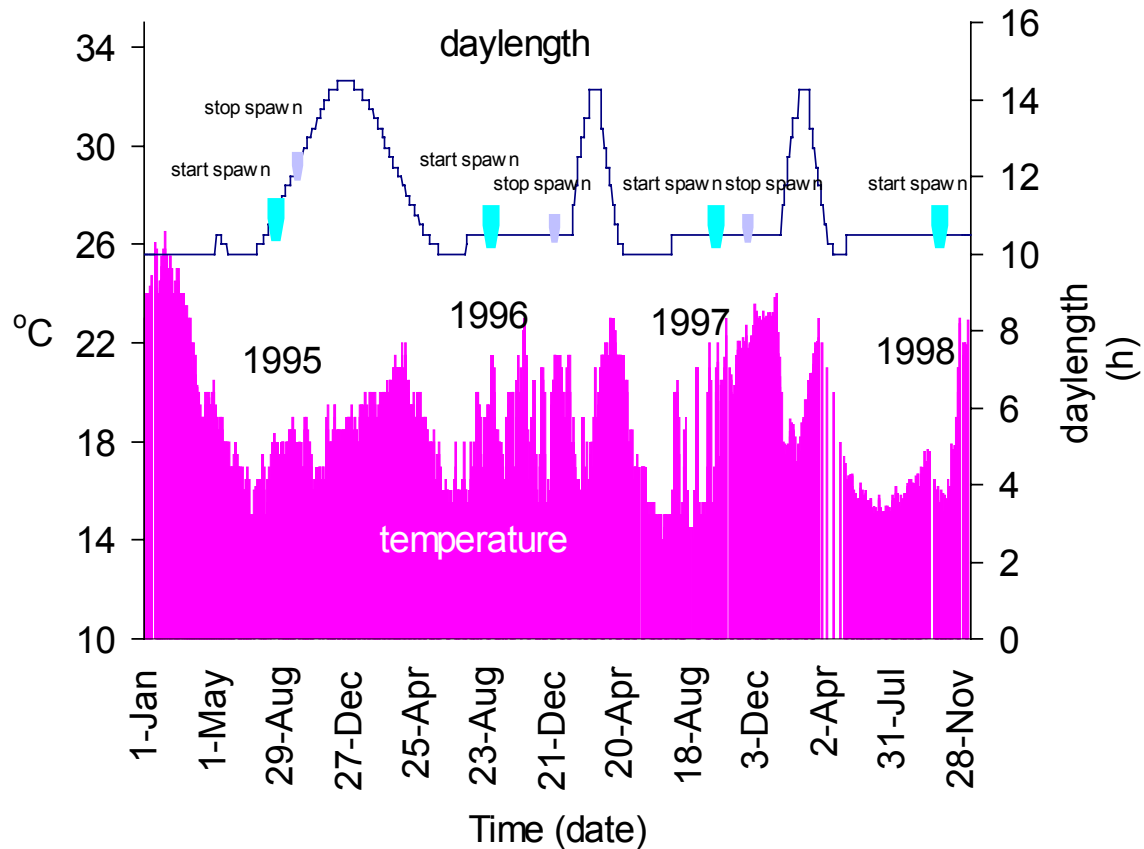


Figure 3. Actual water temperature and photoperiod and spawning events of captive G1 broodstock snapper from 1995 to 1998.

The wild caught snapper did not spawn naturally during the same period. However gonad biopsies of 10 randomly selected fish on 7/8/96 revealed that females were maturing (oocytes 80 - 455 μ m) and 2 males were spermiating. All fish were biopsied on 30/8/96 and 9 females had oocytes with mean largest (n=10) diameters ranging from 125-440 μ m and 8 males were spermiating.

In 1997, the G1 snapper began spontaneous spawning on 27 July after daylength and temperature had been increased from 10.0 to 10.5 h and 16.0 to 19.0 $^{\circ}$ C, respectively. Once spawning had commenced, the photoperiod was kept constant at 10.5 h, and only temperature was altered. Wild-caught snapper did not spawn spontaneously during this period.

G1 snapper ceased spontaneous spawning in early September 1997. On 8 October 1997, the G1 broodstock tank was drained and 6 females (40%) were randomly selected, anaesthetised and a gonad biopsy was taken with a 1 mm diameter plastic cannula. Five (5) of the females had primary oocytes (diameter 124.4 \pm 5 μ m; mean \pm sd), indicating that the fish had either completed spontaneous spawning or had not matured after exposure to the truncated phototherm regime. Observation of spawning fish was extremely difficult due to the tank dynamics, therefore identification of individual spawning females was not possible. One (1) G1 female had oocytes of 418 μ m and was implanted with a cholesterol-based LHRH-a pellet (160 μ g LHRH/kg) to induce spawning. Male fish were not cannulated as approximately 6 fish were observed displaying pre-spawning external colouration at dusk. Two (2) males were spermiating freely when slight pressure was applied to the abdomen. After 8 days, spawning started and continued daily for 10

days. The number of eggs spawned daily ranged from 59,000 to 697,000. Fertilisation rate ranged from 6 to 87% ($51.4 \pm 28\%$; mean \pm sd).

The wild-caught broodstock were cannulated on 11 July and 8 October 1997. In July, 10 females (66%) were randomly selected and 5 fish had maturing oocytes (diameter 289, 292, 314, 357 and 397 μ m). It was not possible to collect a gonad sample from the remaining 5 fish. Four of the maturing fish were implanted with cholesterol LHRH-a pellets ($197.5 \pm 10.6 \mu$ g LHRH/kg) to induce spawning. Spawning commenced 4 days after implantation and continued for 14 days. The number of eggs spawned daily ranged from 62,000 to 430,000. Fertilisation of eggs only occurred on 5 of the 14 spawning events and was very low (0.3 to 8%). This data may suggest that initial oocyte development was insufficient to allow production of high quality eggs; however pre-spawning behaviour of males was not observed, therefore lack of viable males may have been a major contributing factor to the poor fertilisation result.

In October, 6 wild-caught females were randomly selected and cannulated, and 3 fish had oocytes with diameters of 357, 372 and 421 μ m respectively. Three (3) females had primary oocytes only. Three (3) males were assessed for maturity and were each spermiating freely. Two(2) of the mature females were injected with 1000 IU/kg hCG, but spawning did not occur.

3.2. Effect of environmental conditions on sex hormone levels in captive and wild broodstock

Results are presented in Cleary (1997).

3.3. Clearwater intensive larval rearing (PSFC)

3.3.1. Effect of photoperiod on growth and survival of snapper larvae

Experiment 1

There was a significant effect ($P < 0.05$) of photoperiod on growth of snapper larvae from 3-15 dah (Fig. 5). Larvae increased in size and ontogeny as the number of daylight hours was increased. Larvae grown in 0 h and 6 h light did not survive for more than 3 and 6 days, respectively. However, swimbladder inflation of larvae held in 24 h and 18 h light was significantly ($P < 0.05$) lower than larvae held in 12 h light. The experiment was terminated after 12 days as growth of larvae without swimbladders is compromised and quality of larvae is poor.

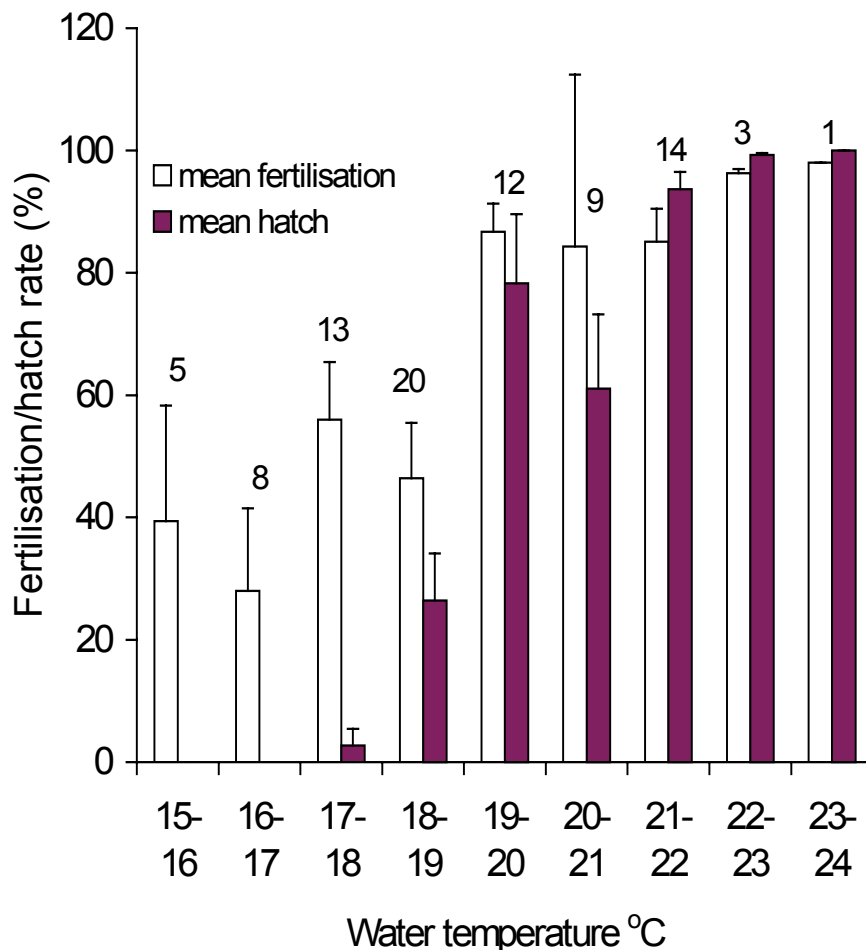


Figure 4. Fertilisation and hatch rates of G1 snapper eggs when naturally spawned at different temperatures during three spawning seasons. Data are means \pm se; n = numbers are spawns.

Experiment 2

Photoperiod significantly ($P < 0.05$) affected growth of snapper larvae. Snapper larvae grown in 18L:6D had final wet weights (32 dah) that were 1.3 and 1.9 times heavier than those grown in 24L:0D and 12L:12D, respectively (Table 1). Mean survival of larvae did not differ between treatments ($P > 0.05$).

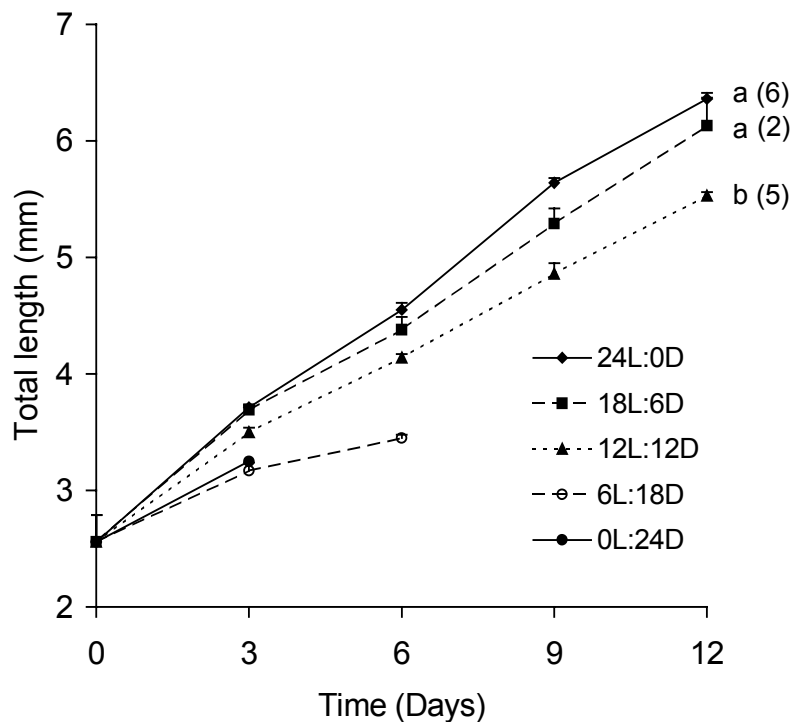


Figure 5. Total length of snapper larvae grown from 3 dah to 15 dah in 100-l tanks under different photoperiod regimes. Data are means \pm s.e. Points with different letters are significantly different ($P < 0.05$); (n) = number of tanks sampled.

Table 1. Final harvest wet and dry weights (mg) of snapper larvae grown from 11 to 32 dah in 100-L tanks and exposed to different daylength regimes. Data are means \pm se for n=5 tanks. Means within columns with different letters are significantly different ($P < 0.05$).

Daylength regime	Final wet Weight (mg)	Final dry Weight (mg)
12L:12D	8.76 \pm 1.02 c	1.46 \pm 0.17 c
18L:6D	16.41 \pm 0.68 a	2.78 \pm 0.13 a
24L:0D	12.70 \pm 0.78 b	2.11 \pm 0.14 b

3.3.2. Effect of temperature on growth and survival of snapper larvae

There was a significant effect ($P < 0.05$) of temperature on growth and survival of snapper larvae. All larvae transferred from 21°C to 30 and 33°C, and 27°C died after 3 and 9 days, respectively (Fig. 6). Larvae survived in 1-2 replicate tanks at temperatures of 15-24°C. There was a significant effect ($P < 0.05$) of temperature on growth of larvae. After 18 days, total length of larvae increased as temperature was increased from 15-24°C. There was a significant effect ($P < 0.05$) of temperature on incidence of urinary calculi. The incidence of calculi occurred earlier and in a greater number of larvae when temperature was increased (Fig. 7). There was a clear reduction in the number of larvae with calculi grown in 24°C from day 18-day 21. The number of larvae with inflated swimbladders was also significantly affected ($P < 0.05$) by temperature (Fig. 8). Larvae grown at 18-24 °C inflated their swimbladders at high and similar rates. However, larvae grown in 15°C and 27°C inflated their swimbladders at significantly slower rates than those grown in 18-24 °C.

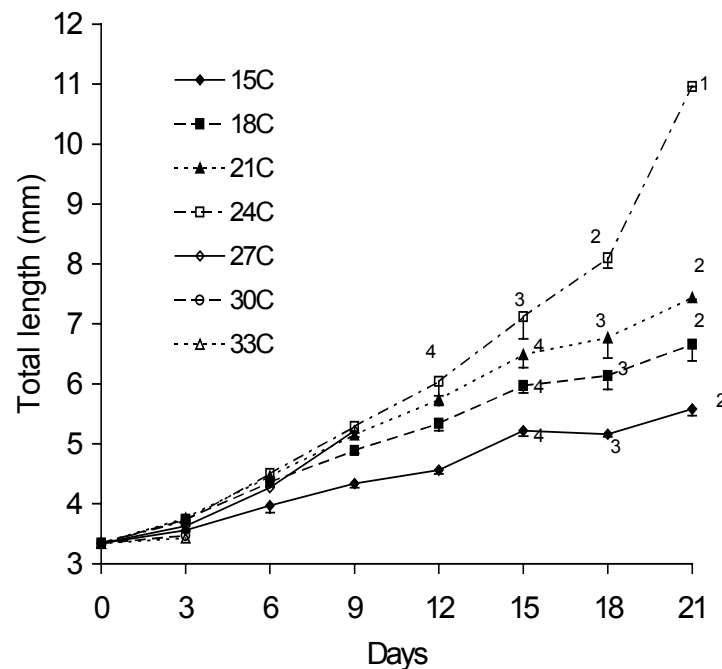


Figure 6. Total length of snapper larvae grown from 3 to 24 dah in different temperatures. Data are means \pm se; n = number of replicate tanks.

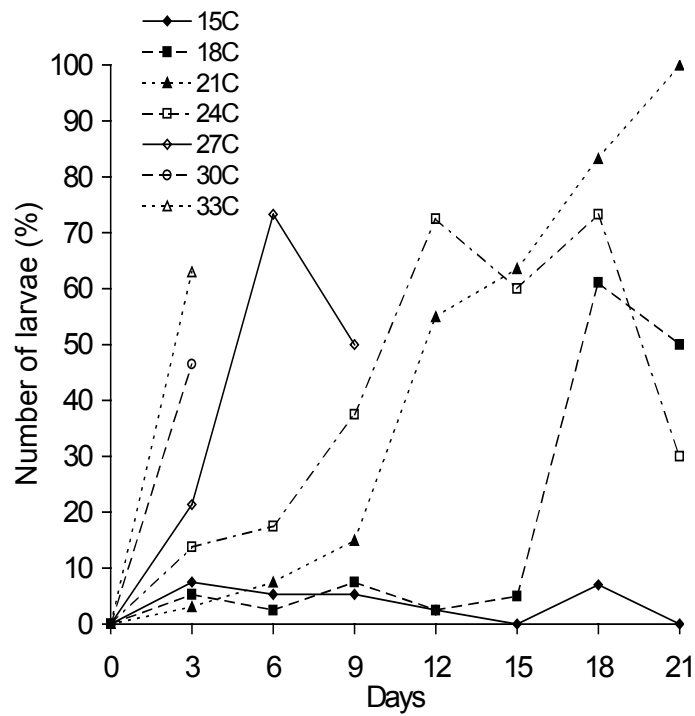


Figure 7. Mean number of snapper larvae with urinary calculi grown from 3 to 24 dah in different temperatures.

3.3.3. Effect of salinity on growth and survival of snapper larvae

There was a significant effect ($P < 0.05$) of salinity on growth and survival of snapper larvae. All snapper larvae held in 5 ppt died within 48 h of transfer from 35-5‰. Some snapper larvae survived for 18 days in all salinities from 10-45‰ (Table 2). Multiple comparison of means failed to clearly identify the optimum salinity for larval survival, however survival appeared best at 20-35‰. Larvae were significantly shorter ($P < 0.05$) at 45‰ than at 10-35‰, which did not differ. There was a significant effect ($P < 0.05$) of salinity on presence of urinary calculi in snapper larvae (Table 2). The incidence of calculi showed a strong relationship between salinity. In general, the number of larvae with calculi increased as salinity was increased. After 18 days the number of larvae grown in 10-25‰ with calculi was low and did not differ, however most larvae grown in 30-45‰ had developed calculi. There was no relationship between incidence of urinary and calculi and survival of snapper larvae. There was no significant effect ($P > 0.05$) of salinity on swimbladder inflation, and ranged from 52.2-86.7% of larvae with inflated swimbladders at day 21 for 10 and 35‰, respectively (Table 2).

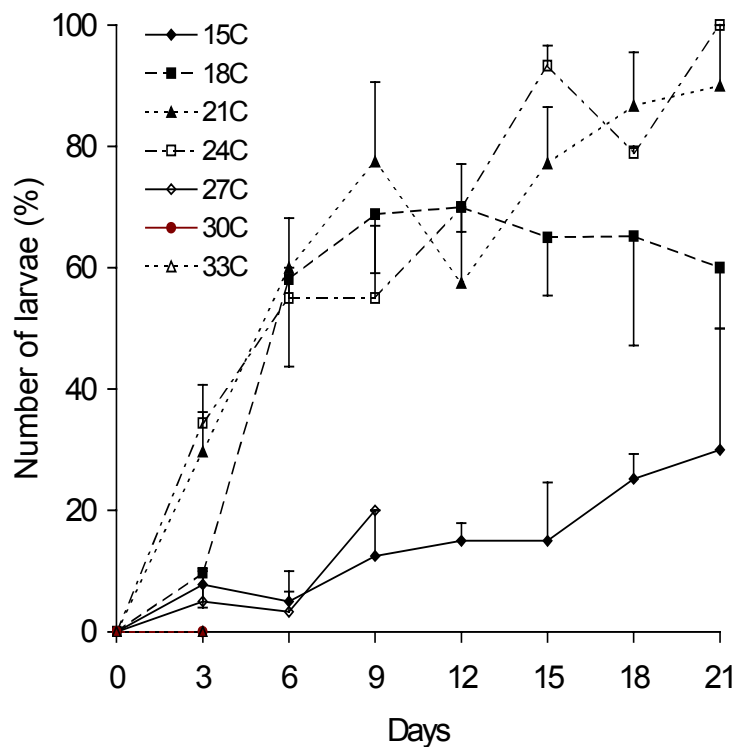


Figure 8. Number of snapper larvae grown from 3 to 24 dah in different temperatures with inflated swimbladders. Data are means \pm se.

3.3.4. *Effect of interaction of temperature and salinity on growth and survival of snapper larvae*

There was no significant effect of salinity or temperature on survival of snapper larvae ($P > 0.05$) (Fig. 9). However at the end of the experiment, the number of replicate tanks with live larvae was significantly greater ($P < 0.05$) for all tanks at 21°C than at 18 or 24°C. Power of the experiment was low (0.1) due to high variability within treatments. There was no significant interaction ($P > 0.05$) between salinity and temperature on final total length of larvae (Fig. 10). Salinity did not affect final total length ($P > 0.05$), however there was a significant effect ($P < 0.05$) of temperature on final total length of larvae. Total length of larvae increased as temperature was increased. There was no significant effect ($P > 0.05$) of salinity or temperature on final swimbladder inflation of larvae. There was a significant interaction ($P < 0.05$) between salinity and temperature on incidence of urinary calculi. Very few larvae grown at salinity of 20‰ developed urinary calculi over the 21 days of the experiment. However significant numbers of larvae grown at 35‰ developed calculi and the incidence increased as temperature was increased. Approximately 100% of larvae grown at 35‰ and 24°C had calculi after 9 days, whereas larvae grown at the same salinity and 21 and 18°C did not achieve 100% incidence of calculi until day 12 and 21, respectively. At day 21, there was a marked reduction in the number of calculi present in larvae grown at 35‰ and 24°C, indicating that size of larvae may affect the ability of calculi to be expelled from the urinary bladder, or that the physiology of the larvae is changing which allows excretion of the calculi.

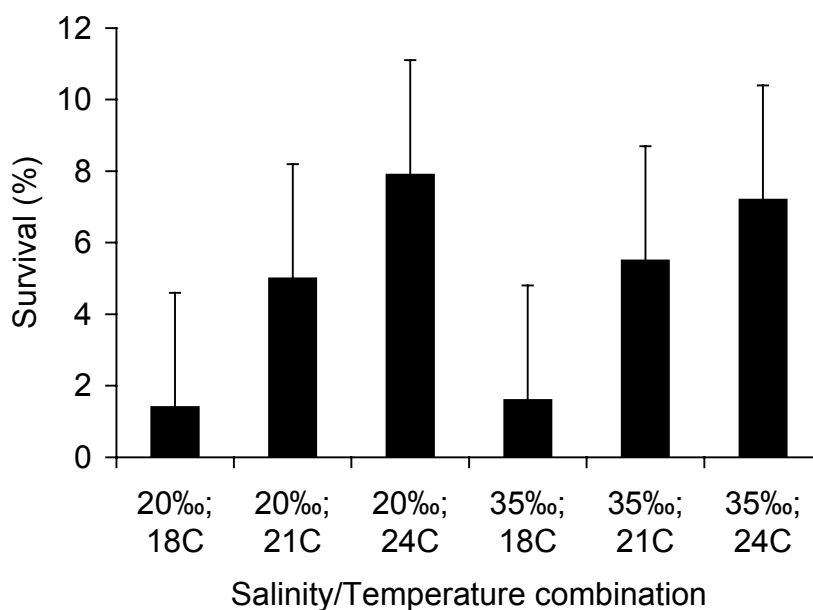


Figure 9. Survival of snapper larvae grown from 3 to 24 dah in different salinity and temperatures. Data are means \pm se ($n = 2$ tanks). Means were not significantly different ($P > 0.05$).

3.3.5. Commercial-scale evaluation of optimum photoperiod, temperature and salinity identified in small-scale experiments

Larvae grown in the new regime of physical parameters grew significantly more quickly than did those in the control regime (Fig. 11). After 30 days, larvae in the new regime were fully weaned onto artificial pellet diet and were 1.4-fold longer (15.6 ± 0.5 mm TL) and 3.3-fold heavier (42.4 ± 3.4 mg wet weight) than unweaned larvae in the control regime (11.1 ± 0.2 mm TL; 12.9 ± 0.8 mg wet weight) (Fig. 12). Survival did not differ between treatments and was $13.3 \pm 1.9\%$ and $14.2 \pm 3.0\%$ for the new and control treatments, respectively.

Based on the growth curve for snapper grown in the control physical parameter regime (Total Length [mm] = $0.2642 \cdot \text{days} + 2.7158$; $R^2 = 0.99$), the predicted time for snapper larvae to reach 15.6 mm TL from 2 dah was 49 days. Therefore, the new regime of physical parameters reduced the time for production of 15.6 mm TL snapper by approximately 40% (19 days).

3.4. Clearwater commercial trials, Pisces Marine Aquaculture (PMA)

Significant mortality syndrome was experienced in several larval batches when larvae were 28 to 30 dah. The companies production target was achieved with approximately 54,000 weaned fingerlings harvested.

3.5. Greenwater larval rearing

3.5.1. Experiment 1 (PSFC)

Table 2. Final percent survival, total length, wet weight, dry weight, incidence of urinary calculi and swimbladder inflation of snapper *Pagrus auratus* larvae grown in different salinities from day 3 to day 21*.

Salinity (‰)	Percent Survival	Total length (mm)	Wet weight (mg)	Dry weight (mg)	Urinary calculi (%)	Inflated swimbladders (%)
10	1.3±0.1 ^a	6.84±0.06 ^a	—	—	0.0±0.0 ^a	52.2±16.1 ^a
15	6.6±2.1 ^{ab}	7.07±0.21 ^a	3.3±0.3 ^a	0.57±0.07 ^a	0.0±0.0 ^a	83.3±6.7 ^a
20	33.4±10.8 ^b	7.13±0.15 ^a	3.3±0.2 ^a	0.55±0.04 ^a	0.0±0.0 ^a	76.7±8.8 ^a
25	29.2±2.8 ^b	6.82±0.09 ^a	2.9±0.1 ^a	0.48±0.03 ^a	10.0±10.0 ^a	76.7±23.3 ^a
30	18.0±6.6 ^b	6.91±0.29 ^a	3.3±0.1 ^a	0.59±0.04 ^a	80.0±5.8 ^b	76.7±3.3 ^a
35	19.7±4.3 ^b	6.88±0.2 ^a	3.0±0.4 ^a	0.52±0.07 ^a	96.7±3.3 ^c	86.7±6.7 ^a
45	3.2±1.7 ^a	5.95±0.26 ^b	—	—	100.0±0.0 ^c	68.5±7.1 ^a

* Data are means ± standard errors for 3 replicate tanks. Within columns, means with a common superscript do not differ (P>0.05).

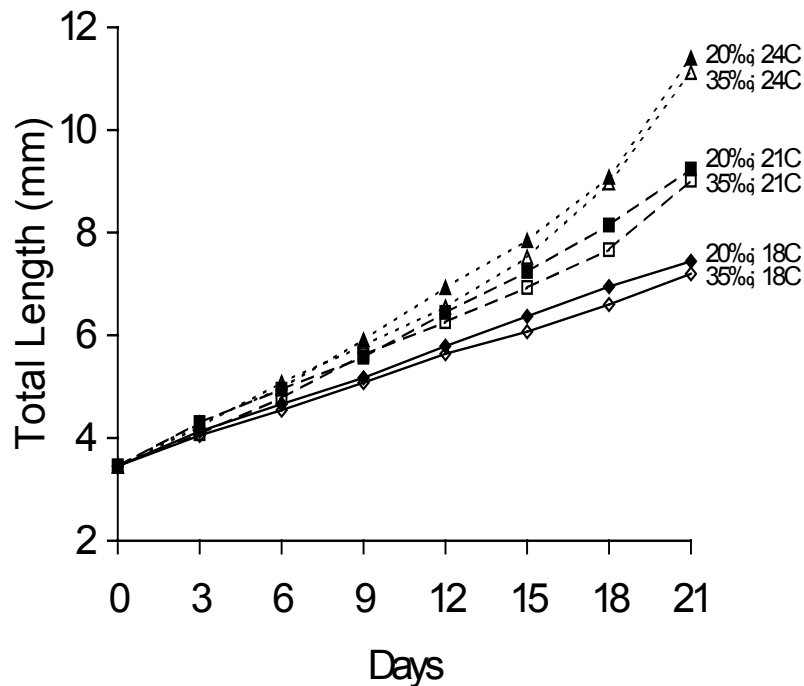


Figure 10. Total length of snapper larvae grown from 3 to 24 dah in different salinity and temperatures. Data are means for n = 5, 4, 3 and 2 tanks for days 3, 6-9, 12-15 and 18-21, respectively.

Larvae grew well during the experiment and readily accepted the artificial diet from 18 dah. Abnormal swimming behaviour and high mortality from 20 to 30 dah, were not experienced during the experiment. This mortality phenomenon has been common with snapper larvae reared at Australian hatcheries in the past. It has been suggested that the mortality syndrome may be due to inadequate nutrition, such as inability for larvae to digest lipids of newly hatched artemia nauplii, and/or HUFA deficiency. Major differences in the feeding regime involved with this experiment compared to previous larval rearing of snapper at PSRC included (1) early introduction of high quality weaning diet and (2) delay in initiation of artemia feeding (typically 12 to 14 dah), no feeding of newly hatched nauplii, and only feeding enriched metanauplii.

At 32 dah, the tanks were drained and larvae harvested. Mean \pm SD survival and total length of larvae (n=3 tanks) were $14.4 \pm 3.0\%$ and $11.5 \pm 0.9\text{mm}$ respectively. Most of the larvae had metamorphosed and were subjectively considered in terms of their evenness in size, swimming vigour and general condition, to be very high quality fish.

Generally, all handled fish (n=10 fish, 3 replicate beakers) from each rearing tank, compared to unhandled control fish, showed immediate signs of stress. Handled fish did not faint but they swam erratically and were not interested in feeding. However after 30 min, nearly all fish (>90%) had regained normal swimming behaviour and commenced vigorous feeding. There was no difference in mortality of control and handled fish after 24 h and was $\leq 10\%$. This data suggests a high degree of quality of the juvenile snapper.

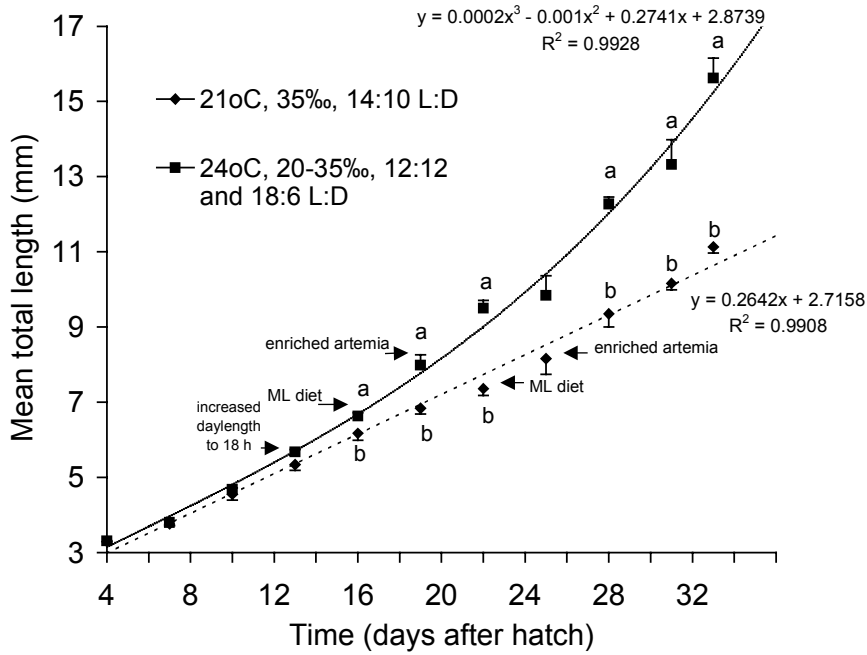


Figure 11. Total length of snapper larvae grown in 2000 l tanks under 2 rearing regimes. Data are mean \pm se for n = 3 tanks. Points with different letters for each time are significantly different ($P < 0.05$).

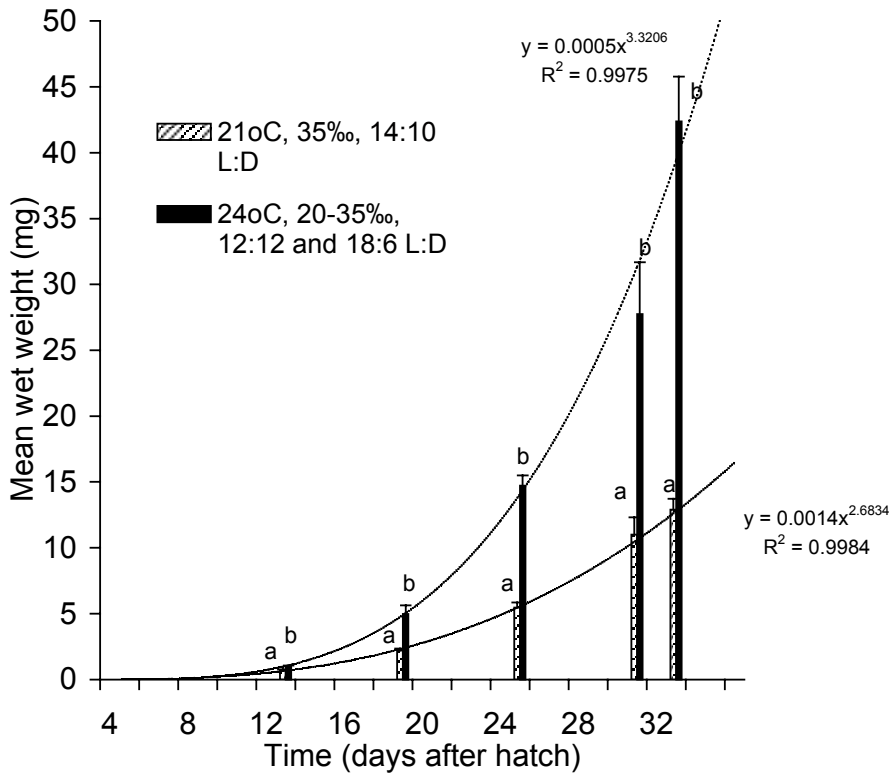


Figure 12. Mean wet weights of snapper larvae grown in 2000 l tanks under 2 rearing regimes. Data are mean \pm se for n = 3 tanks. Points with different letters for each time are significantly different ($P < 0.05$).

3.5.2. *Experiment 2, BIARC*

Swimbladder inflation of larvae was poor. The technique of adding enrichment diet directly to the culture water is likely to be responsible, and is not considered suitable for rearing of snapper larvae. Recent trials carried out in which rotifers were enriched separately instead have shown that swimbladder inflation rates of over 90% can be achieved in greenwater cultures, and poor inflation is not an artefact of the greenwater technique itself.

Results indicate that extensive pond culture of snapper is a viable culture technique. Mean survival of larvae from hatch to day 48 using a 20 day hatchery phase followed by a 28 day pond phase was 25.2% (n =2 ponds). Survival of larvae reared in this manner was not significantly different from that of larvae reared in intensive tank culture to day 30 followed by a 14 day pond phase (24.5%, n = 1). Survival of juveniles from day 30 to day 44 in pond culture was 100%, indicating the adaptability of snapper to pond conditions at this age.

Growth of snapper larvae stocked into ponds at day 20 was slow compared with larvae reared in tanks. Low pond water temperatures limited pond productivity and larval growth. However, the growth of juveniles stocked into pond N1 at day 30 compared very favourably with that in tanks despite lower water temperatures. This, together with the high survival rate and stress-test survival observed in this pond, suggest that fish derived excellent nutritional value from the pond. Hence a pond nursery phase from approximately day 30 may be a very beneficial culture strategy for snapper. Fish stocked into ponds at day 20 were not as hardy at harvest (90% stress test survival). Weakened fish, however, lacked a swimbladder and the lower hardiness is not considered to be a result of the culture method, but the inability of fish with dysfunctional swimbladder to cope with conditions of low water temperature and lower food density.

3.6. **Extensive fertilised pond larval rearing**

3.6.1. *Glen Searle Aquaculture (GSA)*

Experiment 1

After 10 weeks from stocking, ponds were drained but no fish were harvested. Quality of the initial plankton bloom in all ponds was poor (G. Searle, pers. comm.) and was the most likely reason for the lack of survival of larvae.

Experiment 2

No larvae or fingerlings were harvested from the pond after 4 weeks.

Experiment 3

No larvae or fingerlings were harvested from the ponds after 6 weeks.

Experiment 4

A total of 438 and 8 fish with an average weight of 8.4 g (n=50) were harvested from each pond, respectively.

3.6.2. Extensive pond trials (PSFC)

Age of stocking significantly affected ($P < 0.05$) survival of larvae; however, survival was low in all treatments. Larvae stocked at 4 dah had significantly ($P < 0.05$) lower survival ($0.35 \pm 0.49\%$) than those stocked at 8 ($1.8 \pm 0.04\%$) and 16 ($2.1 \pm 1.9\%$) dah, which did not differ. Growth of larvae was rapid (range 0.8-1.0 mm/d) and did not differ between treatments.

3.7. Saline groundwater growout

3.7.1. Small-scale laboratory experiments (PSFC)

Results are presented in Appendix 3.

3.7.2. Pilot-scale pond experiments (WTSSDS)

Trial 1

At 153 d post-stocking, 23 fish were captured. These fish had survived pond temperatures to 8°C but had grown slowly (mean increment 9 g). At 206 d post-stocking, 22 fish were recaptured. The fish had grown significantly (mean increment 71.5 g in 53 d) due to increased water temperature (Fig. 13).

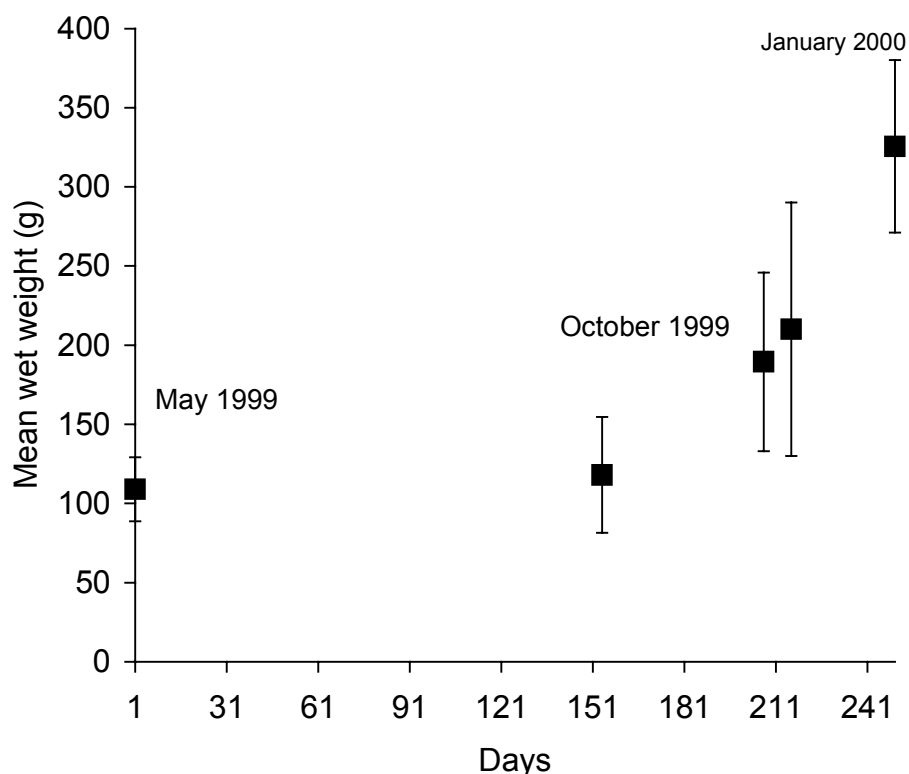


Figure 13. Mean weight of snapper grown in a 0.15 ha pond at the WTSSDS, Wakool. Fish were stocked on 12 May 1999. Data are mean \pm sd ($n = 10-20$ fish).

On 17 January 2000, 9 tagged fish at 332 g (mean increment 1.5g/d), and 4 untagged fish at 310.5 (mean increment 2.8 g/d) were recaptured (Fig. 13). On 19 January, total mortality of fish occurred. The most likely cause was a pond crash due to rapid temperature increment from 14 to 19 January 2000, associated with a die-off of phytoplankton and consequent reduction in dissolved oxygen. Estimated survival from 14 December to 19 January was 98.2%.

Trial 2

No fish died after stocking and many fish were observed feeding vigorously 24 h after stocking. This trend continued for 40 d; however on 14 April, 2000 complete mortality occurred overnight. Due to the rapid nature of the mortality event, the most likely cause was depletion of dissolved oxygen overnight by a dense phytoplankton bloom following three successive days of cloudy weather. Unfortunately, part-time and irregular monitoring of pond water quality did not provide sufficient data to predict a pond crash and to instigate a water exchange program to potentially avoid water quality problems. Until the mortality event, the snapper had grown well (Fig. 14) and no fish died. In addition, the snapper were in excellent condition (~270 g) with light silver to pink external colour.

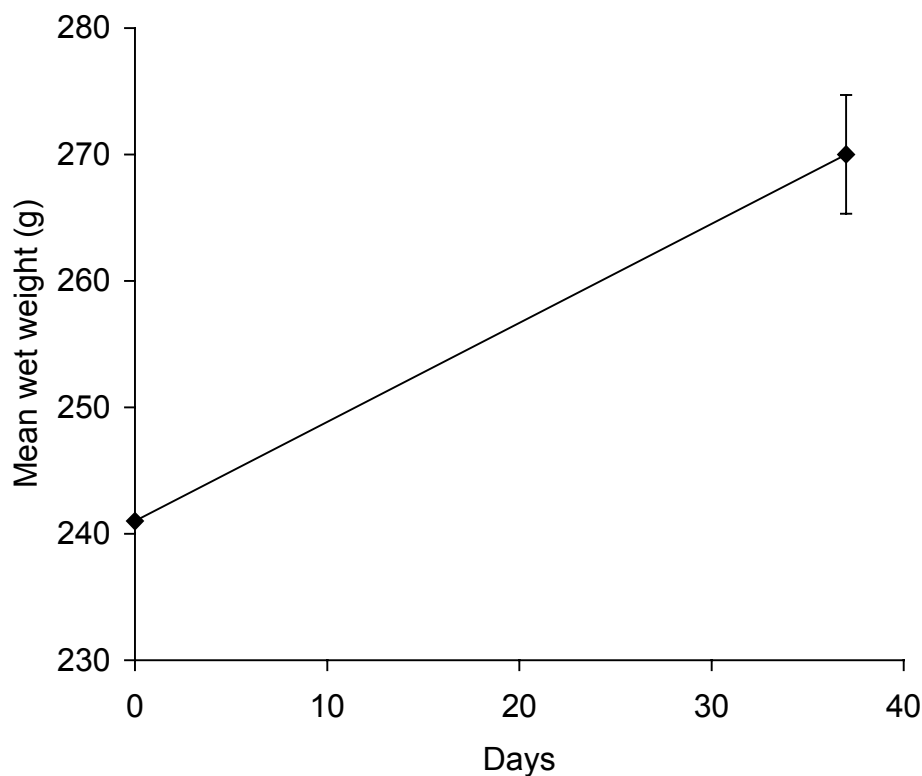


Figure 14. Mean wet weight of snapper grown in a 0.15 ha pond at the WTSSDS, Wakool filled with fortified saline groundwater. Data are mean \pm se for fish ($n = 80$) which were freshly dead on 14 April 00. Fish were stocked on 8 March 00.

Cage Trial

After 43 d, fish were divided evenly into two identical 1 m³ floating cages. Fish grew well for a further 42 d with SGR of approximately 2.3 %/d since stocking. Estimated mean biomass/cage at day 84 (5/1/00) was 3.4 kg/m³. Fish did not grow during the next 14 d and all fish were found dead on the morning of 19 January (day 99; Fig. 15). Survival to day 99 was 96%.

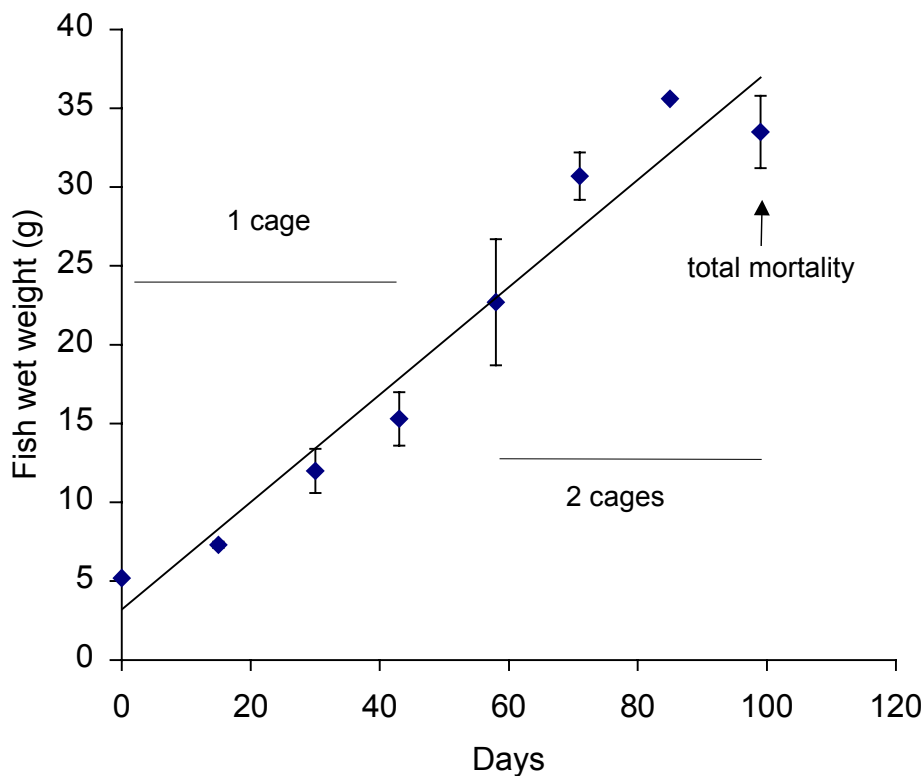


Figure 15. Mean weight of snapper grown in floating cages within a 0.15 ha pond at the WTSSDS, Wakool filled with saline groundwater fortified with potassium. Fish were stocked into cages on 12 October 1999. Data are means \pm sd ($n = 10$ fish for 1 or 2 cages).

3.8. Polytunnel trials (SARDI)

3.8.1. Trial 1

After 10 days the snapper mean wet weight had increased from 1.6 g to 2.8 g (specific growth rate [SGR] - 5.73%). From day 10-20 a thick algal bloom established in the tank and mean wet weight increased from 2.8 g to 4.0 g (SGR 3.52%). This result highlights the need to improve water treatment within the delivery system in order to maintain high water quality required to optimise growth rates to allow profitable culture of fish using saline groundwater. Improved water filtration following pre-treatment of groundwater has since been installed to address this problem. During the final 10 days, mean wet weight increased from 4.0 to 5.8 g (SGR - 3.69%). Overall SGR for 30 days was 4.31%.

Water temperature variations recorded throughout the 30 day growth trial show that the maximum recorded within the recirculating system was 30°C and the minimum was 19°C. The daily difference for water temperature varied between 2 and 5°C with a mean daily difference of 3.4°C. When water temperatures approached 30°C it could be reduced by allowing a small flow of ambient temperature saline groundwater into the recirculating holding system.

At harvest, only 908 fish were recorded of an expected 1584. The most likely explanation was an under estimation of initial post-stocking mortality.

3.8.2. Trial 2

Fish grew from approximately 25 to 400 g in 9 months (Fig. 16). Reports from respected seafood marketers suggest that further improvements can be made to enhance pigmentation. Analysis of 3 of these fish for levels of pesticides and heavy metal indicate that these are not detectable or are below levels allowed by the Health Commission (Food Standard 12 - Metals and Contaminants in Food).

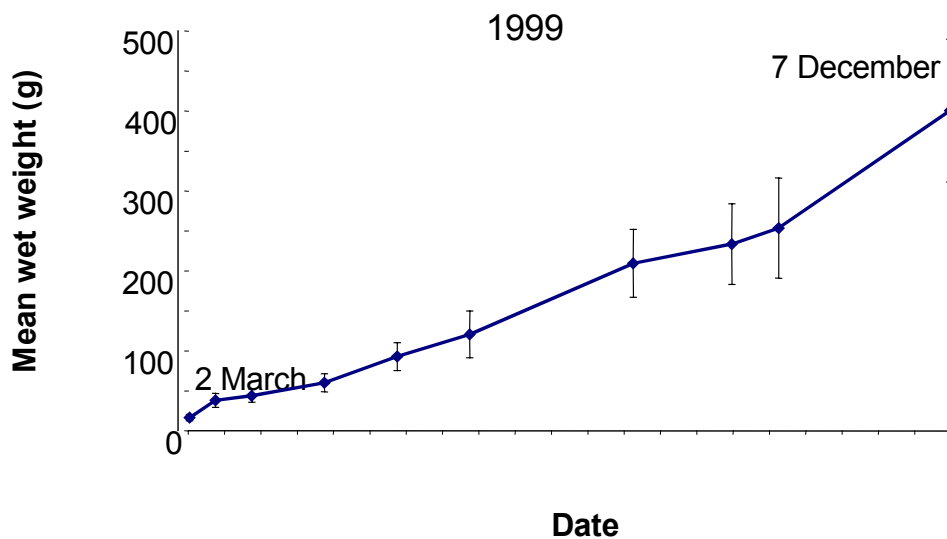


Figure 16. Growth of snapper in saline groundwater at Cookes Plains, SA (March to December 1999).

4. DISCUSSION

Broodstock Management

Land-based broodstock tanks, which included environment (temperature and photoperiod) control and recirculation of seawater through mechanical and biological filters were successfully designed and operated for the duration of the project. Importantly, methods were developed to maintain snapper broodstock in excellent health over many years and it is now possible to spawn snapper all year.

Both wild-caught and First generation hatchery-reared broodstock (G1) snapper matured in tanks after exposure to ambient (365 day) and truncated (120 day) artificially manipulated phototherm regimes. However, the origin of snapper broodstock had a significant effect on spawning reliability and egg quality. Both male and female G1 snapper matured after exposure to 1 ambient and 2 successive truncated phototherm regimes regime and spawned spontaneously when photoperiod and temperature were manipulated. Female G1 snapper did not mature synchronously and spawning occurred over a 5-6 month period. Alternatively, wild-caught snapper did not spawn spontaneously but it was possible to induce spawning with LHRHa.

The quality of eggs and larvae ranged from poor to excellent. Temperature of water at spawning has a major effect on egg development. When spawning occurred between 15.5-18.5°C, development of fertilised eggs was generally abnormal. However, when spawning occurred between 19-23°C, fertilisation rates, egg quality and development, and larvae quality were generally excellent. Both fertilisation and hatching rates of >90% were common. Temperature could also be used to manipulate the frequency of G1 spawning, once spontaneous spawning had started at $\geq 19^\circ\text{C}$. Spawning could be stopped temporarily if water temperature was reduced rapidly to 14-15°C. When temperature was rapidly increased to 19°C, spawning started within 1-2 d.

Baseline (normal) levels of stress and reproductive steroids in captive and wild female Australian snapper were determined, and correlated with reproductive condition. G1 female snapper were found to respond to stress differently than other wild-caught groups. G1 female snapper become stressed during handling; however, they recover quickly and stress steroid levels reduce rapidly. On the other hand, wild-caught snapper had constant, high, stress steroid levels. These results confirm the observations of spontaneous spawning by G1 snapper and the lack of spontaneous spawning by wild-caught snapper in tanks (Cleary et al. 2000).

The most successful protocol for hormonal induction of ovulation was also identified. Reproductive steroid levels decrease dramatically and rapidly (within 6 hours) after capture and confinement stress. The response of snapper to stimulation by exogenous hormones (Lueteinising Hormone Releasing Hormone analogue (LHRHa), human Chorionic Gonadotropin (hCG), 17-hydroxyprogesterone), at two different treatment times (first capture, and 24h post capture) showed that egg quality was better in snapper treated with hCG than those treated with LHRHa. In addition snapper injected at first capture ovulated greater egg volumes, coupled with better quality eggs (Cleary et al. under review).

Clearwater Intensive Larval Rearing

At the start of this study, the “best practice” regime of temperature, salinity and photoperiod for rearing snapper larvae was 21°C, 35‰ and 14L:10D; however, use of this regime was based on experience rather than identification of specific optima. Our study determined the optimum regime

initially in replicated small-scale experiments followed by commercial-scale evaluation of the parameters in large tanks.

Small-scale experiments showed that the optimum regime of temperature, salinity and photoperiod for rearing snapper larvae was different to the original “best-practice” regime and was 24°C, 35-20‰, and 12L:12D to swimbladder inflation then 18L:6D to metamorphosis (new regime), respectively. Comparison of the original and new regimes in a commercial-scale trial showed that growth of larvae was significantly greater under the new regime. After 30 d, larvae grown in the new regime were fully weaned onto artificial pellet diet and were almost 1.4 times longer and almost 3.5 times heavier than larvae grown in the original regime, which were still reliant on live feeds. Based on the growth curve for snapper grown in the original physical parameter regime (Total Length [mm] = 0.2642*days + 2.7158; $R^2 = 0.99$), the predicted time for snapper larvae to reach 15.6 mm TL from 2 dah was 49 days. Therefore, the new regime of physical parameters reduced the time for production of 15.6 mm TL snapper by approximately 40% (19 days).

Our results have major implications for the cost of production of juvenile snapper. A 40% reduction in time to rear snapper larvae to fully-weaned juvenile fish will allow greater turn-over of hatchery production batches resulting in potentially 1.6 times more production of fingerlings. Overhead operating costs of hatchery production will ostensibly remain the same, thus fish will be produced more cheaply.

Greenwater Larval Rearing

Trials to evaluate the suitability of greenwater, intensive rearing of snapper larvae were successfully conducted at PSFC and BIARC. The techniques used at both sites mostly followed those described by Palmer et al. (1992); however, some modification of the techniques was necessary, especially at BIARC to improve swimbladder inflation of larvae. In general, snapper larvae grew well in greenwater tanks and survival of 20-30 dah larvae ranged from 14-25%. Quality of advanced larvae was assessed by observing the rate of recovery and survival of larvae following exposure to handling stress. In general, provided fish had functional swimbladders, the fish quality was high.

Extensive Larval Rearing

Four trials were conducted at Glen Searle Aquaculture (GSA) and one trial at PSFC and BIARC, respectively to evaluate the suitability of extensive fertilised ponds for rearing of snapper larvae. Trials ranged from replicated experiments investigating the effect of age of larvae at stocking on growth and survival to simple, unreplicated pond growth trials, which were opportunistic depending on availability of larvae and ponds.

In general, in trials at GSA and PSFC, survival of larvae stocked at 2-14 dah was low and ranged from 0-2%. Growth of surviving larvae, however, was high at around 0.8-1.0 mm/d. Significant problems were experienced at GSA with poor live food production in ponds prior to stocking first-feeding snapper larvae, coupled with potentially high post-stocking mortality of larvae after transport from PSFC to GSA.

Alternatively, when advanced 20 and 30 dah snapper larvae were stocked into fertilised ponds at BIARC, growth and survival (up to 100%) to fully metamorphosed juveniles were high. These results suggest that the best use of extensive fertilised ponds for larval rearing of snapper may be in combination with an initial three week rearing phase in intensive or greenwater systems.

Extensive rearing of snapper larvae has the potential to significantly decrease the cost of fingerling production due to low overhead costs and high growth. However, there is an essential need to continue evaluation of this technique to identify optimum rearing strategies.

Saline Groundwater Growout

Results of our trials demonstrate that saline groundwater at the WTSSDS is suitable for culture of snapper in ponds provided the groundwater is fortified with potassium. Existing, aged sediments in the evaporation ponds are unsuitable for fish but this can be overcome by using a plastic pond liner. Also, lengthy acclimation is necessary to avoid post-stocking mortality of snapper when juveniles are transported from Port Stephens to Wakool.

Snapper grew well during summer and survived low winter temperatures. Acute mortality events occurred on several occasions and were most likely due to pond crashes or severe oxygen depletion due to excessive phytoplankton blooms. These problems are experienced potentially by all pond aquaculture facilities and can be predicted and prevented by regular water quality monitoring and exchange of water.

There is excellent potential to develop aquaculture of a range of species in saline groundwater at the WTSSDS. However, evaluation of new species and development of pond management practices is necessary in rigorous pilot-scale trials. Therefore it is necessary to expand the experimental facilities at the WTSSDS to provide ponds for replicated experiments and to employ a full-time scientist and Fisheries Technicians to operate the experiments.

Polytunnel Trials (SARDI)

Results of trials conducted at the Cookes Plains Groundwater Interception Scheme (CPGIS) showed that saline groundwater is suitable for growth and survival of juvenile snapper and does not require ionic adjustment. However, the groundwater is high in iron, which must be removed by aeration and filtration before it can be used to culture snapper. Snapper grew well in a recirculation system housed within a polytunnel (greenhouse), which increased minimum winter water temperatures.

There is significant potential to continue development of techniques for culture of snapper in environmentally controlled facilities using saline groundwater from the CPGIS.

5. REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY OF BLACK BREAM *ACANTHOPAGRUS BUTCHERI*

5.1. Background

This part of the project was conducted by PhD student James Haddy under the supervision of Professor Ned Pankhurst, School of Aquaculture, University of Tasmania. James was funded through an Australian Postgraduate Award and adopted as a CRC student in view of the contribution that his work would make to the general question of sparid management in inland saline waters of variable salinity. The project encompassed 4 general areas of study.

5.2. Annual change in reproductive condition and plasma levels of sex steroids

The annual change in reproductive condition and plasma levels of sex steroids in black bream were investigated by measuring reproductive parameters from wild caught fish from 2 estuaries on the east coast of Tasmania. Black bream have an annual cycle with a 3-month spawning season in spring and early summer. Within this, there are daily cycles of gonadal maturation and plasma steroid levels. Elevated levels of plasma 17β -estradiol (E_2), testosterone (T) in females, and T and 11-ketotestosterone (11KT) in males were associated with gonadal recrudescence, and elevated levels of $17,20\beta$ -dihydroxy-4-pregnen-3-one ($17,20\beta$ P) were found in association with final oocyte maturation in females and spermiation in males.

5.3. Stress induced changes in plasma levels of sex steroids

Stress induced changes in plasma steroid levels were investigated by blood sampling fish at capture and after confinement for up to 24 h. Confinement resulted in significantly elevated plasma cortisol levels and reduced plasma T and E_2 levels in females within 1 h of capture. In males, the changes in T and 11KT were seen as little as 30 mins after the imposition of stress. In contrast, plasma levels of $17,20\beta$ P remained unchanged or in some cases, increased. The results confirm that as in other sparids, stress has a rapid and profound inhibitory effect on reproductive endocrine function.

5.4. Efficacy of exogenous hormones at stimulating changes in sex steroid levels and ovulation

The effect of hormone therapy in relation to stress status was assessed by injecting females with human chorionic gonadotropin (hCG) or luteinising hormone releasing hormone analogue (LHRHA) either straight after capture, or following 24 h transport and subsequent confinement. Both hormones stimulated a significant increase in the percentage of females ovulating, with LHRHA at capture producing the best response. Injection of both hormones at capture resulted in elevations in plasma T and E_2 , whereas hormone treatment 24 h post capture did not change steroid levels. Plasma levels of cortisol and $17,20\beta$ P were unaffected by hormone treatment. The results show that capture and handling stress reduce the effectiveness of exogenous hormone treatment, and that for best results, fish need to be treated as soon after capture as possible.

5.5. The effects of salinity on reproductive development, plasma steroid levels, fertilisation and egg survival

Gonadal maturation and seasonal change in steroid levels of captive fish maintained at 5 to 35‰ were unaffected by salinity. Hormone treatment resulted in typical endocrine and ovulatory responses at all three salinities. However, egg production was reduced in fish held at 5‰, and sperm motility and subsequent egg fertility were effectively nil. This shows that while reproductive development can be maintained over a wide salinity range, the salinity window for effective gamete fertilization and egg survival is substantially lower. This will set the boundary limits for reproductive stock maintenance under brackish conditions.

5.6. Publications arising from the study

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7. APPENDICES

7.1. Appendix 1: List of participants

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7.2. Appendix 2: Publications submitted or in preparation from this study

Fielder, D.S., W.L. Bardsley and G.L. Allan. Survival and growth of Australian snapper, *Pagrus auratus* in saline groundwater from inland New South Wales, Australia. (submitted to *Aquaculture* June 2000)

Fielder, D.S., Bardsley, W.J., Allan, G.L., Pankhurst, P.M. Effect of photoperiod on growth and survival of snapper, *Pagrus auratus* larvae. (in preparation)

Fielder, D.S., Bardsley, W.J., Allan, G.L., Pankhurst, P.M. Effect of temperature and salinity on growth and survival of snapper, *Pagrus auratus* larvae. (in preparation)

- 7.3. **Appendix 3: Survival and growth of Australian snapper, *Pagrus auratus* in saline groundwater from inland New South Wales, Australia. *Manuscript submitted to Aquaculture (June 2000).***

Survival and growth of Australian snapper, *Pagrus auratus* in saline groundwater from inland New South Wales, Australia.

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Abstract

Australia has huge resources of inland saline groundwater, which may be suitable for culture of marine fish. This study assessed the suitability of saline groundwater, which was pumped from a shallow aquifer into an evaporation pond near Wakool in western New South Wales, for growth and survival of juvenile snapper *Pagrus auratus*. Five experiments were conducted. The first showed that snapper (31 g) did not commence feeding, lost equilibrium of buoyancy and became moribund within 3 days after transfer from coastal seawater (diluted to 19‰ with rainwater) to saline groundwater (19‰). Potassium concentration of diluted coastal seawater and groundwater (both 19.6‰) was 203 and 9.2 mg l⁻¹, respectively, while the concentration of most other major ions was similar in water from both sources. In the second experiment groundwater of 21‰ salinity was fortified with potassium (as KCl) to provide 25, 50 or 100% of the concentration of potassium found in coastal seawater of 21‰ salinity. Survival and feeding and swimming behaviour of snapper (1.5 g) held in tanks for 8 days were the same in 50 and 100% potassium fortified treatments as in coastal seawater controls. However, snapper held in groundwater fortified with only 25% potassium, or raw saline groundwater became moribund after 4 and 2 days, respectively. During the third 42 d experiment, growth, survival and food conversion of juvenile snapper (4.0 g) were the same in diluted coastal seawater (20‰) and groundwater (20‰) provided the level of potassium in the groundwater was increased to within 60-100% of the concentration in coastal seawater. During the fourth experiment, juvenile snapper were acclimatised to raw saline groundwater by transferring fish from fortified groundwater with initial potassium levels of 100% of that in coastal seawater, to groundwater with 10% lower potassium levels every 3.5 days or 20% lower levels every 7 days. A further treatment where snapper were transferred from groundwater fortified initially with potassium levels of 60% of coastal seawater, to groundwater with 20% lower potassium levels every 3.5 days was included. When potassium was reduced to 20% of the concentration in coastal seawater, in all treatments, fish became moribund. Results from the fifth experiment, where groundwater was fortified with either KCl or NaCl at equivalent chloride levels, confirmed that potassium and not chloride ions were responsible for improvement in groundwater. Our results demonstrate that saline groundwater from Wakool, fortified with KCl is a suitable medium for growing snapper juveniles in tanks.

Keywords: Snapper, *Pagrus auratus*, Aquaculture, Saline groundwater

1. Introduction

Snapper, *Pagrus auratus*, occurs in temperate waters in all Australian states, Lord Howe Island and Norfolk Island (Battaglione and Bell, 1991) and is the target of recreational and commercial fisheries (Bell et al., 1991; Francis, 1994). Juveniles usually live in estuaries, while adults inhabit coastal and offshore waters. Catches, particularly from the Australian east-coast are declining (ABARE, 1998) and as a consequence programs to develop aquaculture protocols for snapper are current in temperate Australia (Battaglione and Fielder, 1997).

Australian snapper is the same species as the Japanese red sea bream (*P. auratus* = *P. major*; Paulin, 1990), which has been cultured successfully in Japan for 30 years, using intensive larval rearing followed by growout in sea-cages (Foscarini, 1988; Battaglione and Bell, 1991). It is seen as an excellent candidate species for aquaculture and possibly stock enhancement in Australia.

Commercial snapper hatcheries and floating sea-cage farms are now operating in New South Wales (NSW), South Australia and Queensland using techniques described by Battaglione and Talbot (1992), Battaglione (1995) and Quartararo (1996) or adapted from those used in Japan to rear red sea bream. However, expansion of a sea-cage-based snapper farming industry in Australia may be limited by the lack of sites with suitable water quality, water depth and proximity to land-based infrastructure (Ogburn, 1996). Approval of sea-cage farms by relevant government bodies is also affected by conflict with other waterway users and perceived concerns about environmental impact.

Alternative, additional sites may be available for farming marine species if the large reserves of saline groundwater occurring in inland areas of Australia can be utilised. Shallow saline water tables are migrating towards the soil surface in many semi-arid areas especially where crops are irrigated (Blackwell, 1999). Crop production is affected adversely and natural vegetation is destroyed. In some inland areas upward migration of the water table is addressed by pumping saline groundwater into a series of large evaporation ponds, which range in size from 2-30 ha (Ingram et al., 1996; Allan and Fielder, 1999). The salt concentration of this water increases progressively as it flows from one pond to the next and eventually crystalline salt is deposited.

The largest saline groundwater evaporation scheme in Australia is the Wakool-Tullakool Sub-Surface Drainage Scheme at Wakool, NSW (35°28'S, 144°26'E), where approximately 13,000 Ml yr⁻¹ of saline groundwater is pumped into 1,600 ha of evaporation ponds (Ruello, 1996). The major ions found in seawater, in order of magnitude, are chloride (Cl⁻), sodium (Na⁺), sulphate (SO₄²⁻), magnesium (Mg²⁺), calcium (Ca²⁺) and potassium (K⁺) (Spotte, 1979). In saline groundwater from Wakool, the concentration of Cl⁻, Na⁺, SO₄²⁻ and Mg²⁺, is similar to seawater of the same salinity; however, the concentration of Ca²⁺ and K⁺ in Wakool saline groundwater is much higher and lower, respectively, than seawater of the same salinity.

Saline groundwater has been used successfully in the United States and the Middle East to culture a range of algae, crustaceans and finfish such as tilapia, red drum, sea bream, eels and channel catfish (Forsberg et al., 1996; Ingram et al., 1996; Samocha et al., 1998). In Australia, saline groundwater from shallow and deep aquifers has been suitable for growth and survival of euryhaline finfish such as silver perch, *Bidyanus bidyanus*, Australian bass, *Macquaria novemaculeata*, barramundi, *Lates calcarifer* and Atlantic salmon, *Salmo salar* (Allan and Fielder, 1999; Gooley et al., 1999). To date, attempts have not been made to culture relatively stenohaline marine finfish like snapper in Australian saline groundwater.

The aim of this study was to assess the suitability of saline groundwater collected from an evaporation pond at Wakool, for growth and survival of juvenile snapper.

2. Materials and Methods

Five laboratory bioassays were conducted at the NSW Fisheries Port Stephens Fisheries Centre (PSFC) from July 1997 to April 1998.

2.1. Source of juvenile snapper

Fertilised snapper eggs were obtained from first generation hatchery-reared (G1) snapper held in tanks at PSFC. Snapper either spawned spontaneously or were induced to spawn using hormones (Lee et al., 1986).

The first group of juvenile snapper was produced after larvae were cultured for 57 days in a 2,000-l tank using techniques similar to those described by Battaglione and Talbot (1992). After this time, approximately 1,000 juvenile snapper were harvested from the tank and 200 were placed into each of five, 500-l floating cages placed within a 100,000-l, outdoor, in-ground concrete tank. While in the cages, juvenile snapper were fed a 45% protein, 1-4 mm crumble diet (Kinta Pty Ltd, Yarrowonga, Victoria, Australia). Ambient estuarine water (range, 23-33‰, 14-25°C) was pumped into the tank for 4 h each day to replace approximately 20% of the total tank volume.

The second group of juvenile snapper was produced after larvae were cultured for 32 days in three 10,000-l outdoor, concrete tanks using “greenwater” culture techniques. Each tank was filled initially with sterilised estuarine seawater (32‰, 22.5°C), fertilised with 35 g of inorganic fertiliser (Aquasol[®], Hortico Australia Pty Ltd., Homebush, Australia) and inoculated with 1,500-l of the algae *Nannochloropsis oculata*. The tanks were mostly static with new algae added as required (range 4-19% total tank volume) to maintain an algae density of approximately 1×10^5 cells ml⁻¹. The larval feeding regime was: rotifers, *Brachionus plicatilis*, at 5 individuals ml⁻¹ from 3-32 days after hatch (dah); artemia at 0.2-1 individuals ml⁻¹ from 24-32 dah; and 100 and 200 µm, 54% protein “ML powered” weaning pellet (Nippon Formula Feed Manufacturing Co., Ltd., Tsuruyacho, Kanagawa-ku, Yokohama, Japan) from 18-32 dah. Rotifers and artemia (> 48 h old) were nutritionally enriched with the algae *Pavlova lutheri* and Tahitian *Isochrysis* aff. *galbana*, and DHA Super Selco (Inve Aquaculture NV, Oeverstraat 7-01200, Baasrode, Belgium) for 24 h before harvest.

At 32 dah, juvenile snapper were harvested from the concrete larval rearing tanks and ongrown in 10,000-l fibreglass tanks until used in experiments. Estuarine seawater (30-35‰) was supplied constantly with a flow-through rate of approximately 200 l h⁻¹. While in the tanks, juvenile snapper were fed 400 and 800 µm, 52% protein “ML powered” weaning diet.

2.2. Source of saline groundwater and coastal seawater

Saline groundwater was collected on two occasions from an individual 30 ha evaporation pond which was part of the Murray Irrigation Limited, Wakool-Tullakool Sub-Surface Drainage Scheme (35°28'S, 144°26'E) and transported in tanks by road to the PSFC. The saline groundwater collected first (collection 1) had a salinity of 19‰ and was stored until needed for experiments in 10 x 30-l drums. The salinity increased to 21‰ following evaporation during this study. The saline groundwater collected second (collection 2) had a salinity of 30‰ and was stored in a 10,000-l storage tank which was covered by a lid to exclude light and reduce evaporation.

Coastal seawater was collected from an ocean beach at Port Stephens (32°45'S, 152°04'E), transported by road to the PSFC and stored in a 10,000-l tank.

Generally, the chemistry of the saline groundwater (SG) from Wakool and coastal seawater (CS) of the same salinity was similar (Table 1). Both SG and CS were dominated by chloride ions, which constituted approximately 56% of the total salinity. Of the other major elements, in order of magnitude, the concentrations of sodium and sulphate in SG were 80% of that in CS, whereas the

concentrations of magnesium and calcium were 1.5 and 2.5 times greater, respectively in SG than in CS. There was a major difference in the concentration of potassium, which was present in SG at only 4.5% of the concentration in the same salinity CS. There were mostly small differences in concentration of minor elements, such as heavy metals, in SG and CS (Table 1).

2.3. *Experiment 1: effect of raw saline groundwater*

This experiment was designed to determine the effect of raw saline groundwater on short-term survival and behaviour of juvenile snapper. Two water treatments were tested: raw saline groundwater (SG) and coastal seawater (CS).

The experiment was conducted in 6 x 60-l glass aquaria ($n = 3$ per treatment). Each aquarium was divided (approximately 1:5) by a partition of 300 μm mesh. A simple biofilter consisting of approximately 50 bioballs (Academy Plastics, Minto, NSW, Australia) was positioned in the narrow section of the tank. An airlift, made from a 50 mm pvc tube was located in the middle of the bioballs and moved water through the biofilter and returned it to the larger tank section which contained the experimental fish. Tanks were covered with black plastic to reduce light intensity, but a window covered by a black plastic flap, allowed observation of experimental fish.

Three randomly positioned tanks were filled with each experimental treatment solution (Table 2). Each tank was then stocked with four randomly selected juvenile snapper (mean wet weight 31.3 g, $n = 10$; group 1), which had been acclimatised from 30 to 19‰, 24 h earlier. No water was exchanged during the experiment, which was terminated after 4 days.

2.4. *Experiment 2: short-term effect of fortified saline groundwater*

This experiment was designed to determine the effect of fortifying raw saline groundwater with K^+ (as KCl) on short-term survival and behaviour of juvenile snapper. For this and subsequent experiments, stock solutions for experimental treatments which included K^+ fortification, were prepared by dissolving analytical grade KCl to give the required K^+ concentration (Table 2). Coastal seawater was diluted using rainwater. The following water treatments (all 21‰) were tested:

- (a) raw saline groundwater (SG raw)
- (b) 100% K^+ fortified saline groundwater (SG100)
- (c) 50% K^+ fortified saline groundwater (SG50)
- (d) 25% K^+ fortified saline groundwater (SG25)
- (e) coastal seawater control (CS).

The experiment was conducted in 15 x 2-l opaque, plastic buckets with lids ($n = 3$ buckets per treatment). A total of 1.5 l of each experimental water treatment was placed into three randomly selected buckets. Approximately 85% of the bucket water volume was poured to waste each day and replaced with new treatment water. Air was supplied to each container through a 1-ml glass pipette at 50 ml min^{-1} . Fluorescent light was provided at the surface of each container at 3.0 $\mu\text{mol s}^{-1} \text{m}^{-2}$ on a 14:10 h light:dark photoperiod.

Each bucket was stocked with 4 randomly selected 61 dah juvenile snapper (mean wet weight 1.5 g, $n = 4$; group 2), which had been acclimatised from 30 to 21‰, 24 h earlier. The experiment was terminated after 8 days.

2.5. Experiment 3: long-term effect of fortified saline groundwater

This experiment was designed to determine the effect of fortifying raw saline groundwater with K^+ on long-term survival and growth of juvenile snapper. The water treatments (all 20‰, unless otherwise stated; Table 2) tested were:

- (a) 100% K^+ fortified saline groundwater (SG100)
- (b) 80% K^+ fortified saline groundwater (SG80)
- (c) 60% K^+ fortified saline groundwater (SG80)
- (d) 40% K^+ fortified saline groundwater (SG40)
- (e) 100% K^+ fortified saline groundwater, 30‰ (SG100-30)
- (f) coastal seawater control (CS).

The experiment was conducted in 24 x 100-l conical bottomed tanks with black sides and white bottoms ($\underline{n} = 4$ tanks per treatment). Each tank was part of an independent, recirculating system operated with an internal 500- μm mesh-covered standpipe, an external airlift pump and biofilter and an internal biofilter. The external biofilters were filled with approximately 1.5-l of shell-grit with particle surface area of $0.79 \pm 0.44 \text{ cm}^2$ (mean \pm s.d.; $\underline{n} = 20$). The shell-grit was covered with a 1-cm layer of filter wool, which collected detritus. Water was pumped through the external biofilter at 2-3 l min^{-1} (120-180% tank volume h^{-1}). The internal biofilter consisted of a 2.75-l (350 mm long x 100 mm diameter) pvc pipe which was filled with bioballs, retained within the pipe by a piece of 4 mm nylon mesh secured at both ends, which also prevented fish from swimming into the biofilter. Air was provided at approximately 5-l min^{-1} by an airstone, which was attached to the bottom of the filter. The filter was immersed completely within the experimental tank. Incandescent light was provided at the surface of each tank at $23 \pm 1.3 \mu\text{mol s}^{-1} \text{ m}^{-2}$ (mean \pm s.e.; $\underline{n} = 30$ tanks) on a 14:10 h light:dark photoperiod. Approximately 5% of the experimental tank water was exchanged each day and salinity was maintained by adding rain water when needed.

Each experimental water treatment was placed into four randomly selected tanks. Juvenile snapper (97 dah; group 2) were anaesthetised with 20 mg l^{-1} of benzocaine in the stock tank, and a random sample of fish was weighed to provide an estimate of fish weight ($3.9 \pm 0.8 \text{ g}$, \pm s.d., $\underline{n} = 20$). Fish were then weighed individually and 6 fish of similar size were stocked into each experimental tank. Fish were fed to satiation daily by hand at 0900 and 1500 h with 52% protein “ML powered” weaning pellets. The experiment was terminated after 42 d.

2.6. Experiment 4: acclimation of juveniles to raw saline groundwater

This experiment was designed to determine if juvenile snapper could be acclimatised from KCl fortified saline groundwater to raw saline groundwater by rapid or slow dilution of the K^+ concentration. The acclimation treatments (all 21.5‰; Table 2) tested were:

- (a) 100% K^+ fortified saline groundwater; no reduction in K^+ (SG100-C)
- (b) 100% K^+ fortified saline groundwater; K^+ reduced by 10% every 3.5 days (SG100-slow)
- (c) 100% K^+ fortified saline groundwater; K^+ reduced by 20% every 7 days (SG100-rapid)
- (d) 60% K^+ fortified saline groundwater; K^+ reduced by 20% every 7 days (SG60-rapid).

The experimental buckets, daily water management and lighting for this experiment were the same as those described for Experiment 2. Each experimental water treatment was placed into three randomly selected buckets. Three randomly selected 109 dah juvenile snapper (mean wet weight $3.2 \pm 0.6 \text{ g}$, \pm s.d., $n = 10$; group 2) were then placed into each bucket. The experiment was terminated after 33 days.

2.7. Experiment 5: effect of K^+ or Cl^-

This experiment was designed to determine whether the improvement in performance of juvenile snapper in raw saline groundwater fortified with KCl was due to an increase in K^+ or Cl^- concentration. The water treatments (21‰; Table 2) tested were:

- (a) 100% K^+ fortified saline groundwater (SGK100)
- (b) saline groundwater fortified with analytical grade NaCl to provide the same Cl^- concentration as occurred in treatment (a) (SGCL100).

The experiment was conducted in 6 x 4-l glass beakers ($\underline{n} = 3$ beakers per treatment), each filled with 3.4-l. The daily water management and lighting were the same as those described for Experiment 2. Each experimental water treatment was placed into three randomly selected beakers. Each tank was then stocked with 3 randomly selected 168 dah juvenile snapper (mean wet weight 21.9 ± 4.5 g, \pm s.d., $\underline{n} = 6$ tanks; group 2). The experiment was terminated after 4 days.

2.8. Measurement of survival, fish behaviour, growth and feeding

For experiments 1, 2, 4 and 5 snapper were fed to satiation by hand with 54% protein “ML powered” pellets once each day at approximately 0900 h when the number of surviving fish and the number of fish feeding were counted, and swimming behaviour was assessed. Moribund and dead fish were removed from the tanks as soon as they were noticed. In experiments 2, 4, and 5 the feeding and swimming behaviour of individual fish was scored on a scale of 5 (normal) to 1 (abnormal).

In experiment 3, all moribund and dead fish were removed from the tanks as soon as they were noticed. Stocking density was maintained in each tank by replacing dead fish with fin-clipped fish of similar weight. A group of replacement fish for this purpose were pelvic fin-clipped for identification 7 d before the start of the experiment, treated for 48 h with 50-100 mg l^{-1} of oxytetracycline hydrochloride to prevent infection of wounds and then held in a 400-l tank with flow-through estuarine seawater (30-32‰). Replacement fish were not used in estimates of weight gain. Feed consumption was recorded daily. Weight gain was recorded every 14 d. After 42 d, fish were harvested and the percentage survival, mean fish wet weight, mean adjusted biomass gain = [final total biomass + weight of dead fish] – [initial total biomass + weight of replacement fish], mean fish dry weight and food conversion ratio (FCR) were calculated from data recorded for each tank.

2.9. Daily water measurement

In all experiments salinity, temperature, pH and dissolved oxygen were measured daily using a water quality meter (Horiba U-10, Horiba Ltd, Kyoto, Japan) (Table 3). In Experiment 3, Total ammonia-nitrogen (< 1.0 mg l^{-1}) was measured daily in at least one replicate tank from each treatment with a rapid test kit (E. Merck, Model 1.08024, 64271, Darmstadt, Germany). Light intensity (photosynthetically active radiation) was measured with a light meter (LI-COR, model Li-1776, Lincoln, NE, USA).

2.10. Statistical analyses

Data were assessed for homogeneity of variance using Cochran's test (\underline{C} ; Winer, 1971). For Experiment 3, data for survival ($\underline{P} = 0.0001$, $\underline{C} = 0.8$) and FCR ($\underline{P} = 0.003$, $\underline{C} = 0.7$) were heterogeneous and could not be transformed to satisfy the assumption of homogeneity of variance. Experiments 2-5 were designed for analysis using single factor analysis of variance (ANOVA). Where significant differences were found, means were compared by the Student-Newman-Keuls test (SNK). Statistical analyses were conducted using Statgraphics Version 5.0 (STSC Inc., Rockville, MD, USA).

3. Results

3.1. Experiment 1: effect of raw saline groundwater

Snapper held in SG were actively swimming for 1 d following transfer from CS, but did not feed vigorously. After 2 d, one fish had died and two fish had lost equilibrium of buoyancy and were floating upside down, while the remaining fish were lethargic and displayed no flight response when challenged with external stimuli. This trend continued, and after 4 d all fish in the SG were removed from treatment tanks following loss of equilibrium of buoyancy, or had died. Snapper held in CS treatments for 4 d all survived, and were actively swimming and feeding during this time.

Effort was made in this and all subsequent experiments to remove moribund snapper from the treatment solutions and soon as they were noticed, and return them to coastal seawater; however, some snapper died prior to removal from treatment solutions and no fish survived after transfer to seawater.

3.2. Experiment 2: short-term effect of fortified saline groundwater

After 8 d, all fish that were held in CS, SG50 and SG100 treatments had survived and there was no significant difference ($P > 0.05$) in swimming and feeding behaviour (Table 4). No fish survived for more than 2 and 4 days when held in the SG raw and SG25 treatments, respectively.

3.3. Experiment 3: long-term effect of fortified saline groundwater

Survival of snapper was high and not significantly different ($P > 0.05$) for all water treatments (Table 5). A total of three fish died during the experiment as a result of jumping from the tanks; one fish in one tank of the SG60 treatment, and two fish in one tank of the SG40 treatment.

Snapper grew in all water treatments; however, the final wet weight of snapper grown in the SG40 treatment, was significantly lower ($P < 0.05$) than that of snapper grown in CS, SG60, SG80, SG100 or SG100-30, which did not differ (Table 5). Multiple comparisons of means for final dry weight failed to clearly separate treatment differences; however, the final dry weight of snapper grown in SG40 was significantly ($P < 0.05$) lower than that of snapper grown in SG60, SG80, SG100 or SG100-30 which were similar (Table 5).

The amount of feed consumed and FCR were affected significantly ($P < 0.05$) by the concentration of K^+ in the saline groundwater; however, multiple comparisons of the means failed to clearly separate the main treatment effects. The fish grown in the SG40 consumed up to 28.8% less feed than snapper grown in the SG100 treatment. Feed consumption was similar for the other treatments (Table 5). The FCR of snapper was low and similar for fish grown in the CS, SG60, SG80, SG100 and SG100-30 treatments, but was higher for snapper grown in the SG40 treatment (Table 5).

3.4. Experiment 4: acclimation of juveniles to raw saline groundwater

All snapper survived and the feeding and swimming behaviour of the fish were similar and normal for all acclimation treatments provided the K^+ concentration was $\geq 40\%$ of that in equivalent salinity CS (Fig. 1). However, when the K^+ concentration was diluted to 20% for the SG100-slow, SG100-rapid and SG60-rapid treatments, some fish lost equilibrium and stopped feeding within 1-2 d and all fish were moribund after 4-6 d.

3.5. Experiment 5: effect of K^+ or Cl^-

Snapper that were held in the NaCl fortified groundwater treatment began to lose equilibrium of buoyancy and appetite after 1 d following transfer from coastal seawater. After 2 d, four fish were moribund and the remaining fish were lethargic and did not feed well. This trend continued, and after 4 d all fish in the NaCl fortified groundwater treatment were moribund. Snapper held in the KCl fortified groundwater treatment for 4 d all survived, and were actively swimming and feeding during this time.

4. Discussion

Raw saline groundwater from Wakool was not suitable for survival and growth of juvenile snapper. Within 1-2 d following transfer of snapper from coastal seawater (CS) to raw saline groundwater (SG), fish started to lose equilibrium of buoyancy, floated upside down and did not feed well. The performance of snapper did not improve with time and by approximately 4 d after transfer all snapper were moribund or dead.

The concentration of the major ions, Cl^- , Na^+ , SO_4^{2-} , Mg^{2+} and Ca^{2+} in SG was either similar to or greater than that of equivalent salinity CS. However, the concentration of K^+ in the SG was extremely low, being only 4.5% (9.2 mg l^{-1} ; 19.6‰) of that available in equivalent salinity CS (203 mg l^{-1}). The salinity and chemistry of saline groundwater can vary widely. For example, in a similar study in Texas, USA saline groundwater was collected from 35 sites and representative samples evaluated for their suitability for aquaculture of red drum, *Sciaenops ocellatus* (Forsberg et al., 1996). The concentration of the major ions were: salinity, 2–35‰; Cl^- , 639–15,443 mg l^{-1} ; Na^+ , 537–9,403 mg l^{-1} ; SO_4^{2-} , 164–5,934 mg l^{-1} ; Mg^{2+} , 8–1,263 mg l^{-1} ; K^+ , 5–87 mg l^{-1} ; and Ca^{2+} , 36–1,179 mg l^{-1} .

Sodium, potassium and chloride are essential minerals for animals due to their role in electrolyte and acid-base balance (Wilson and El Naggar, 1992). Potassium is the main cation of intracellular fluids while sodium and chloride are the major extracellular ions. Osmoregulation, or the maintenance of constant extracellular and intracellular osmolality, is mostly determined by the homeostasis of these ions (Teeter, 1997). Fish can readily derive all or a portion of these minerals from the water by unidirectional diffusion across the gills and the gut or they can be obtained from food (Shearer, 1988; Lall, 1989; Wilson and El Naggar, 1992). Because fish can sequester potassium from the water, it has been difficult to conduct studies to determine dietary requirement of this ion without altering the ionic composition of the water (Wilson and El Naggar, 1992). Consequently, there is a paucity of information describing the quantitative requirement of potassium (Lall, 1989).

The estuarine Japanese red sea bream, the same species as snapper (Paulin, 1990), and the freshwater channel catfish did not require dietary potassium when grown in seawater and freshwater (4 mg l^{-1} K^+), respectively (Sakomoto and Yone, 1978; Wilson and El Naggar, 1992), thus indicating that these fish had obtained all necessary potassium from the water. Alternatively, Shearer (1988) showed that juvenile chinook salmon, *Oncorhynchus tshawytscha* grown in freshwater with a potassium concentration of < 1 mg l^{-1} , were unable to sequester sufficient potassium from the water and required a dietary potassium concentration of 0.8% for maximum growth. Wilson and El Naggar (1992) however contend that 0.8% is an overestimate of the potassium requirement for chinook salmon. Potassium deficient chinook salmon initially displayed reduced growth rate due to anorexia and poor food conversion and ultimately fish died.

Results of our study showed that performance of snapper improved dramatically when KCl was added to the SG. The enhanced performance of fish was due to an increase in the concentration of potassium ions rather than an increase in the concentration of chloride ions (Experiment 5). In

Experiment 3, growth, feed consumption and FCR after 42 d were the same for snapper held in CS and SG (both 20‰), provided the potassium concentration of the SG was fortified to 60–100% (~ 124–207 mg l⁻¹ K⁺) of the potassium concentration as occurred in equivalent salinity CS. Also, the performance of snapper grown in higher salinity SG (30‰) and fortified to provide 100% (~ 310 mg l⁻¹ K⁺) was the same as the CS control fish. However, snapper held in SG (20‰) fortified with 40% (~ 83 mg l⁻¹ K⁺) of the potassium concentration as occurred in equivalent salinity CS, weighed significantly less ($P < 0.05$) and consumed almost 30% less feed and had a higher FCR than other treatments. Survival of snapper was not affected by these concentrations of potassium in SG; however, below 40% fortification of SG with potassium, fish were moribund within 2–6 d (Experiments 2 and 4).

Fortification of culture water with salts has improved growth and/or survival of fish in several other studies; however results have varied with salt and water type. Survival of red drum was markedly improved from 0 to 93% when the calcium and chloride concentrations of saline groundwater (3–4‰) were increased from 36 to 337 mg l⁻¹, and 639 to 1,296 mg l⁻¹, respectively following addition of CaCl₂; whereas addition of NaCl to the saline groundwater only improved survival slightly (Forsberg et al., 1996). On the other hand, addition of NaCl to freshwater and diluted seawater (1‰) resulted in much better survival (> 2-fold increase) of red drum than when CaCl₂ was added (Thomas and Wolters, 1992; Stahl et al., 1995). Clearly, the efficacy of remediating water by adding salt is dependent on the source and chemical composition of the water.

The symptoms of reduced growth and feed conversion and/or death displayed by snapper in SG with low potassium concentrations are similar to those of chinook salmon fed potassium deficient diets (Shearer, 1988) and also for euryhaline species such as red drum, which were cultured in hypotonic water (Gatlin et al., 1992). In water with low ionic concentration, it is likely that there is a substantial loss of ions from the fish to the water. Osmoregulation, therefore requires increased expenditure of metabolic energy, which may result in reduced fish performance (Bryan et al., 1988). We did not investigate the effects of low potassium concentration in SG on blood osmolality of snapper; however further research in this area is warranted.

Snapper grown in SG fortified with 40% of the potassium concentration as occurred in equivalent salinity CS continued feeding during the experiment and it is likely therefore that some potassium was being derived from the feed. We did not determine the potassium concentration in the feed, which was a high protein, fish meal-based diet. Fish meal is typically a poor source of potassium; however, soybean meal, which constituted 12% of the diet, contains in excess of 2% potassium (Lall, 1989). It is likely therefore that the diet contained at least 0.24% potassium. This diet concentration is similar to the potassium requirement level reported for the channel catfish, 0.26 % (Wilson and El Nagggar, 1992) and some terrestrial animals such as rats, 0.23 % (Bieri, 1977) and pigs, 0.26% (Jensen et al., 1961). Clearly, the potassium concentration in the diet fed in our study did not provide adequate compensation for maximum growth of snapper grown in SG fortified with 40% potassium.

In general, dietary salt (NaCl) supplementation has resulted in little or no improvement in growth and feed conversion of some diadromous species such as Atlantic salmon grown in freshwater and seawater (Shaw et al., 1975), and rainbow trout, *Oncorhynchus mykiss* grown in freshwater (MacLeod, 1978). However, weight gain and food conversion of the euryhaline red drum grown in freshwater was improved when the diet was supplemented with NaCl at 2% or with both NaCl and KCl at 2% each (Gatlin et al., 1992). In our saline groundwater where potassium is deficient, improvements in performance of snapper may be achieved by supplementing the diet with KCl. This should be investigated.

It was not possible to acclimatise juvenile snapper to raw SG by gradually diluting SG fortified initially with either 60 or 100% of the potassium concentration as occurred in equivalent salinity

CS. Regardless of dilution schedule, all snapper became moribund or died when the potassium concentration in the SG (20‰) was diluted to 20% (~ 40 mg l⁻¹) of the potassium concentration as occurred in equivalent salinity CS. Clearly, snapper were unable to compensate for potassium concentration in the SG of approximately 40 mg l⁻¹ or lower. There is very little information available on the physiological and morphological responses of teleosts, in particular marine-adapted fish, to low external ion concentrations. Perry and Wood (1985) showed that calcium uptake was higher in trout, *Salmo gairdneri* when they were held in water with low calcium concentration, compared to when they were held in water with high calcium concentration, and this was correlated with a proliferation of lamellar chloride cells. When Mozambique tilapia, *Oreochromis mossambicus* were held in magnesium deficient freshwater, magnesium deficiency in the fish coincided with increased calcium and sodium content and a low potassium content of the body. An increase in opercula chloride cell density was found also in magnesium deficient water (Bijvelds et al., 1997). Although there was no apparent difference in the performance of snapper grown in SG fortified with 60–100% of the potassium concentration as occurred in equivalent salinity CS, morphological and physiological changes may have occurred, particularly in response to lower potassium concentration in this range. Further research is warranted to determine if snapper compensate physically to SG that is partially deficient in potassium and also if different (e.g. longer) dilution schedules allow snapper to acclimatise to raw SG.

Our results suggest that for normal snapper performance and osmoregulation, SG (20‰) from Wakool must be fortified with potassium to provide a minimum concentration of approximately 120 mg l⁻¹. Because salinity of the groundwater can vary, and the interactions between ions during osmoregulation are complex, it may be more important to consider the ratio of ions rather than the specific concentration of individual ions in the water. Forsberg et al. (1996) suggested that the survival of red drum grown in saline groundwater was correlated with K⁺/Cl⁻ and Na⁺/K⁺ ratios; however, the correlations resulted from within-treatment variation where the highest (100%) and lowest (70%) survival occurred in replicates of the same groundwater treatment. Regardless of this, mean survival was high (85-100%) and instantaneous growth was the same for red drum grown in four different saline groundwaters, which were not fortified with potassium, suggesting that potassium was not deficient. In these authors' experiments, the K⁺/Cl⁻ ratios of saline groundwater (15‰) and the artificial seawater control ranged from 0.007-0.014, and 0.022, respectively. In our study, the K⁺/Cl⁻ ratios of SG treatment water ranged from 0.001 for raw SG to 0.018 for 100% fortified SG. Survival and growth of snapper was achieved in SG provided the K⁺/Cl⁻ ratio was greater than 0.007, but maximum growth was achieved when the K⁺/Cl⁻ ratio was greater than 0.01. When the K⁺/Cl⁻ ratio was less than 0.007, snapper died.

5. Conclusion

Saline groundwater from Wakool was suitable for the culture of snapper in laboratory experiments provided the potassium concentration was fortified to supply a K⁺/Cl⁻ ratio of 0.007-0.018. This was achieved easily by adding KCl to the saline groundwater. Following these results, a pilot-scale project has been established to evaluate the suitability of the saline groundwater for culture of snapper in ponds at Wakool. Further research should also investigate the potential to enhance growth of snapper in partially potassium deficient saline groundwater by adding potassium to the diet. Research should also be conducted to determine if snapper change morphologically and/or physiologically in response to suboptimal concentration of potassium in the saline groundwater.

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Table 1. Water chemistry of coastal seawater and saline groundwater from the Wakool-Tullakool Sub Surface Drainage Scheme, New South Wales, Australia.

Element or Chemical	Coastal seawater		Coastal seawater diluted ¹		Raw groundwater	
Salinity (‰)	35.3		19.6		19.6	
pH	8.1		--		7.9	
Alkalinity, (mg l ⁻¹) as CaCO ₃	114		63		195	
	<i>ion/Cl</i>		<i>ion/Cl</i>		<i>ion/Cl</i>	
<u>Major ions</u>	(mg l ⁻¹)	ratio	(mg l ⁻¹)	ratio	(mg l ⁻¹)	ratio
Chloride	20000	1.000	11105	1.000	11000	1.000
Sodium	9470	0.474	5258	0.474	4210	0.383
Sulphate	2500	0.125	1388	0.125	1100	0.100
Magnesium	1000	0.050	555	0.050	820	0.075
Potassium	365	0.018	203	0.018	9.2	0.001
Calcium	364	0.018	202	0.018	504	0.046
	<u>Minor elements</u>					
Fluoride	0.76		0.42		0.44	
Total PO ₄ , (mg l ⁻¹) as P	< 0.10		--		< 0.10	
Aluminium	< 0.001		--		0.007	
Boron	4.4		2.4		0.38	
Bromide	72		40		34	
Copper	0.012		0.007		0.007	
Iodide	< 0.05		--		0.16	
Iron	< 0.05		--		< 0.05	
Lead	< 0.001		--		< 0.001	
Lithium	0.17		0.09		0.07	
Manganese	< 0.001		--		0.001	
Mercury	< 0.001		--		< 0.001	
Molybdenum	0.012		0.007		0.002	
Strontium	7.6		4.2		9.1	
Zinc	0.004		0.002		< 0.001	

¹values calculated from coastal seawater at 35.3‰.

Table 2. Experimental water treatments, target ion concentrations and quantities of added salts (KCl; NaCl). Coastal seawater (CS) and saline groundwater (SG) were collected from Port Stephens and Wakool, New South Wales, Australia, respectively.

	Treatment	K ⁺ concentration (mg l ⁻¹)	Amount of KCl added (mg l ⁻¹)		
Experiment 1 (19.0 ^b ‰)	CS ^a	197	0		
	SG raw	9	0		
Experiment 2 (21.0 ^b ‰)	CS ^a	217	0		
	SG100	217	395		
	SG50	109	188		
	SG25	54	84		
	SG raw	10	0		
Experiment 3 (20.0 ^c ‰)	CS ^a	207	0		
	SG100	207	378		
	SG80	166	300		
	SG60	124	220		
	SG40	83	141		
	SG100-30 ^d	310	565		
Experiment 4 (21.5 ^c ‰)	SG100	223	407		
	SG90	200	363		
	SG80	178	321		
	SG70	156	279		
	SG60	134	237		
	SG50	112	195		
	SG40	90	153		
	SG30	67	109		
	SG20	45	67		
	SG10	22	23		
	SG raw	10	0		
		K ⁺ concentration (mg l ⁻¹)	added Cl ⁻ concentration (mg l ⁻¹)	Amount of KCl added (mg l ⁻¹)	Amount of NaCl added (mg l ⁻¹)
Experiment 5 (21.0 ^c ‰)	SGK100	207	188	395	0
	SGCL100	10	188	0	310

^aAnalytical reagent grade (Sigma, P-3911).

^bAnalytical reagent grade (Sigma, S-9888).

^cOceanic seawater diluted with rainwater from 35.3 ‰.

^dUndiluted saline groundwater; collection 1.

^eSaline groundwater diluted with rainwater from 30 ‰; collection 2.

^fUndiluted saline groundwater; collection 2.

Table 3. Ranges of water quality parameters for Experiments 1-5.

Experiment	DO ₂ (mg l ⁻¹)	Salinity (‰)	pH	Temperature (°C)
1	5.6-6.4	18.0-19.0	8.3-8.5	20.5-21.6
2	4.0-6.0	20.8-21.5	7.4-8.7	20.3-24.2
3	6.2-7.9	19.7-20.9 (target 20) 29.7-31.1 (target 30)	7.4-8.1	22.1-24.4
4	4.0-7.5	21.5-23.3	7.7-8.1	21.9-22.7
5	5.1-6.9	21.1-21.7	7.7-8.1	21.9-22.6

Table 4. Final survival, swimming and feeding behaviour^a of juvenile snapper *Pagrus auratus* held for 8 d in saline groundwater fortified with KCl to provide potassium at different concentrations as occurred in equivalent salinity coastal seawater (Experiment 2).

Treatment	Survival (%)	Swimming behaviour	Feeding behaviour
Coastal Seawater	100 ± 0	5.0 ± 0	4.8 ± 0.3
SG 100% K ⁺	100 ± 0	4.8 ± 0.3	4.7 ± 0.3
SG 50% K ⁺	100 ± 0	4.8 ± 0.3	4.1 ± 0.5
SG 25% K ⁺	0 ± 0	0 ± 0	0 ± 0
SG raw	0 ± 0	0 ± 0	0 ± 0

Data are means ± standard errors (\underline{n} = 3 tanks).

^a feeding and swimming behaviour of individual fish was scored on a scale of 5 (normal) to 1 (abnormal).

Table 5. Growth performance, survival and food conversion of juvenile snapper Pagrus auratus grown for 42 d in saline groundwater fortified with KCl to provide potassium at different concentrations as occurred in equivalent salinity coastal seawater (Experiment 3)^a.

Treatment	Salinity (‰)	Initial wet weight (g)	Final wet weight (g)	Final dry weight (g)	Adjusted wet weight gain ^b (g)	Survival (%)	Feed input ^c (g)	FCR ^d
Seawater (CS)	20	4.1 ± 0.1 ^x	12.5 ± 0.6 ^x	3.8 ± 0.2 ^y	51.0 ± 3.4 ^x	100.0 ± 0.0 ^x	73.0 ± 1.6 ^{yz}	1.5 ± 0.1 ^{xy}
100% K ⁺ (SG100)	20	4.1 ± 0.1 ^x	14.8 ± 0.6 ^x	4.6 ± 0.2 ^z	64.5 ± 4.2 ^x	100.0 ± 0.0 ^x	78.7 ± 2.6 ^z	1.2 ± 0.04 ^x
80% K ⁺ (SG80)	20	4.0 ± 0.2 ^x	13.9 ± 0.5 ^x	4.2 ± 0.2 ^{yz}	59.5 ± 1.5 ^x	100.0 ± 0.0 ^x	76.4 ± 2.6 ^{yz}	1.3 ± 0.03 ^{xy}
60% K ⁺ (SG60)	20	4.0 ± 0.1 ^x	14.0 ± 0.6 ^x	4.3 ± 0.2 ^{yz}	58.9 ± 2.4 ^x	95.8 ± 4.1 ^x	77.9 ± 2.9 ^z	1.3 ± 0.03 ^{xy}
40% K ⁺ (SG40)	20	3.9 ± 0.2 ^x	10.1 ± 0.8 ^y	2.8 ± 0.2 ^x	35.1 ± 4.8 ^y	91.7 ± 8.3 ^x	56.1 ± 4.2 ^x	1.7 ± 0.2 ^y
100% K ⁺ (SG100-30)	30	3.8 ± 0.1 ^x	12.3 ± 0.5 ^x	3.6 ± 0.2 ^y	51.1 ± 3.4 ^x	100.0 ± 0.0 ^x	66.3 ± 2.6 ^y	1.3 ± 0.1 ^{xy}

^a Data are means ± s.e. for 4 replicate tanks. Means in each column with a different superscript are significantly different ($P < 0.05$).

^b Adjusted wet weight gain = [final total weight + weight of mortalities] - [initial total weight + weight of replacement fish].

^c Mean total feed consumed per tank expressed as grams dry weight.

^d Food conversion ratio = weight of feed fed/adjusted wet weight fish gain.

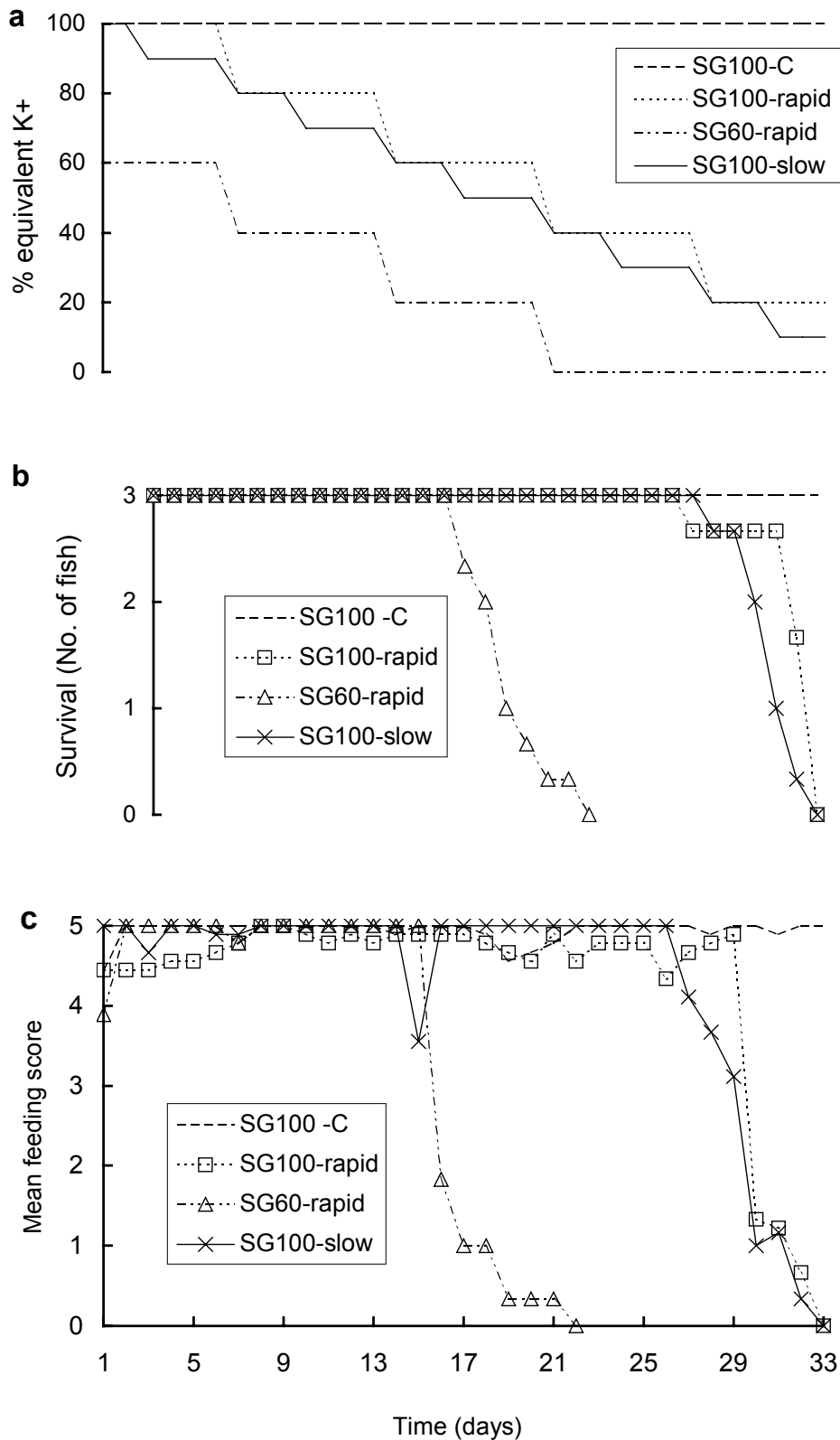


Figure 1. Potassium dilution schedule [a], mean survival [b] and mean feeding score [c] of juvenile snapper *Pagrus auratus* exposed to decreasing concentration of potassium in saline groundwater from Wakool. For survival, data are means of $\bar{n} = 3$ buckets (3 fish per bucket). For feeding, fish were scored from 5 (normal) to 1 (abnormal). (Experiment 4).

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