Aquaculture

Feasibility Study on the Establishment of Harlequin Fish (*Othos dentex*) Aquaculture in South Australia

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PIRSA Innovation Solution and FRDC Project Executive Report

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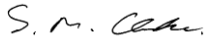
Signed:	
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EXECUTIVE REPORT

Introduction

Harlequin fish, *Othos dentex*, are endemic to Australia and occur from Victoria to Western Australia, with South Australia (SA) central to its natural geographic range (Rimmer *et al*., 2004; Bryars, 2010; Saunders *et al*., 2010). This species has a colourful appearance and appealing texture and taste. According to the preliminary assessment of our industry client, who has more than 15 years experience in promoting and marketing unique fish species in China, this species will fetch a high market price. The longer term benefit of this project is the potential development of a harlequin fish aquaculture industry in SA. This has the potential to enhance regional economies and employment in SA and through diversification to decrease business risk for existing aquaculturists. Hatchery production will also provide opportunities for wild stock enhancement through reseeding in the future if needed because this species is rare in the wild in SA and Victoria (Bryars, 2010; Saunders *et al*., 2010).

This project investigates the key parameters required to assess the potential to develop harlequin fish aquaculture in SA. This includes the potential to establish captive spawning broodstock, the ability of this species to be maintained in production systems and information on the species growth rate. The availability of basic biological information on the harlequin fish will also assist with better management of wild populations, which in some locations are believed to be of conservation concern (Bryars *et al*., 2012).

Fish Collections

During 2011, four trips were undertaken to Kangaroo Island to capture live harlequin fish: 15 March (6 fish), 28 March (11 fish), 29 March (12 fish) and 27 June (8 fish). Fish were captured from a wide range of sites to minimise localised depletion of site-attached populations (Bryars *et al*., 2012); it is likely that collection of large numbers of long-lived broodstock from the same site would be unsustainable in the long-term. After capture, each fish was held in one of two 200 L tanks that were secured on the deck of the vessel. Each tank was supplied with seawater using a 12 v bilge pump positioned over the side of the vessel to pump water through the holding tank with water overflowing into the sea via a flexible hose. On return to Cape Jervis fish were captured using a knotless net and stocked into a 2,000 L transport tank filled with filtered seawater. Oxygen was supplied to this tank from a gas cylinder with a regulator and carbon stone diffuser in the tank. During transportation to the South Australian Aquatic Sciences Centre (SAASC), West Beach (some 100 km and 2.5 hours drive) dissolved oxygen was monitored every 30 minutes. At the SAASC fish were netted into 50 L tubs holding 20 L of seawater and these used to carry fish and stock them randomly into one of two 10,000 L holding tanks. Of the 37 harlequin fish collected, 34 survived the collection and transportation and 3 died during or very soon after these activities.

Fish Maintenance

After initial adaptation of the harlequin fish to the captive environment for one week, attempts were made to induce a feeding response. Individual Australian sardines (*Sardinops sagax*), used as southern bluefin tuna (*Thunnus maccoyii*) feed, were tied using a slip knot to a lightly weighted fishing line and moved around the fish to encourage them to feed. The first captured harlequin fish ate a sardine on 22 March 2011, approximately three weeks after capture. The remaining fish commenced feeding over the following two weeks. The fish then fed voluntarily, including moving toward the project staff at feeding time. The fish were fed twice a week at about 3.5% of total body weight over autumn, with this reduced to about 1.5% over winter. Increasing the feeding frequency to three times a week in autumn did not increase the total amount of feed consumed each week. Attempts to train fish to feed on shrimp, squid and 9 mm yellowtail kingfish pellets (Skretting Australia) were unsuccessful.

External crustacean parasites (Order Copepoda) were found on the skin of some harlequin fish when they were caught in the field. These were caligiform type parasites, probably of the genus *Caligus*. The number of parasites per fish increased substantially in the first month of captive holding. Consequently on 21 April 2011 a preliminary formalin bath treatment of one fish was conducted. One fish was netted from the holding tank and transferred into a tub containing 100 L seawater and 15 mL^{-1} formalin (37% formaldehyde), providing a concentration of 150 mg L^{-1}. The parasites were observed to become active and then drop off the fish after 20 minutes. After 30 minutes in the bath 2 mL anaesthetic (20 mg L^{-1} Aqui-S, 540 g L^{-1} isoeuglenol) was added and after 3-4 minutes the fish was sedated to a level that allowed it to be removed so that the skin surface, gills and mouth could be inspected for parasites. No parasites were found and the fish was returned to the holding tank for recovery. The following day all fish in the holding tank were treated by decreasing the water volume within the holding tank to 2,000 L and adding 300 mL of formalin. Water flow was stopped and aeration increased for 30 minutes before the tank was refilled and returned to normal operating conditions. This prophylactic treatment provided effective control with no parasites subsequently observed on the captive fish.

In October 2011, concrete blocks were used to build shelters on the tank floor as divers have observed apparently paired harlequin fish associated with ‘hides’ in reef structures. Following the installation of shelters, fish changed behaviour from gathering together at the tank floor or beside the inlet plumbing, to hiding in the shelter structures. However, some fish were no longer seen to move out from the shelters for feeding, which made fish monitoring difficult.

Preliminary Assessments of Fish Reproduction and Age

Assessment of the stage of reproductive development of captive harlequin fish was undertaken on 26 October 2011. The fish was first anaesthetized with 5 mL Aqui-S in a 200 L tank. When sedated the fish were removed, measured (mm) and weighed (g). Their sex and reproductive status was assessed by abdominal massage. If no milt was running from the genital pore, a gonad biopsy was taken using a pipelle de cornier (Laboratoire CCD, France) inserted into the gonad to obtain a sample of tissue for

visual assessment. Tissue samples were stored in 10% formalin in case further analysis would be required. In total 12 fish were examined. Oocyte samples were obtained from three females and a small amount of milt ran from the genital pore of one male fish.

Reproductive data presented in Table 1 were collected from 31 of the harlequin fish available from this project. Of these fish, 14 were male, 16 were female and one could not be sexed, suggesting approximately a 1:1 sex ratio. Their total length and weight ranged from 270 mm to 595 mm and 743 g to 2,996 g, respectively (Table 1).

Table 1. Summary of data collected in this study.

Sample No.	Sampling date	Total Length (mm)	Weight (g)	Age	Sex	Gonad Wt (g)	GSI	Rep. Stage*
1	29-Mar-11	595	2996	28	F	75.6	2.52%	2
2	4-Apr-11	530	2206	19	M	2.1	0.10%	2
3	10-Apr-11	412	992	10	F	10.2	1.03%	2
4	10-Apr-11	420	993	9	F	10.2	1.03%	2
5	10-Apr-11	508	1857	19	F	31.9	1.72%	2
6	10-Apr-11	500	1878	18	F	43.9	2.34%	2
7	10-Apr-11	501	1783	18	F	28.4	1.59%	2
8	10-Apr-11	464	1375	11	F	22.0	1.60%	2
9	10-Apr-11	401	938	10	M	<1.0		1
10	10-Apr-11	387	781	9	M	<1.0		1
11	10-Apr-11	373	743	8	M	<1.0		1
12	10-Apr-11	506	2061	25	NA			
13	10-Apr-11	403	922	8	M			1
14	10-Apr-11	505	1922	19	M	<2.0		1
15	10-Apr-11	512	2011	21	M	<1.0		1
16	10-Apr-11	410	850	7	M	<1.0		1
17	7-Jul-11	497	1498	25	M	<1.0		1
18	13-Jul-11	370	745	6	F	5.1	0.68%	1
19	25-Aug-11	535	2830	18	M	1.5	0.05%	1
20	25-Aug-11	421	852	9	M	<1.0		1
21	4-Dec-11	502	1766	19	F	55.0	3.11%	4
22[1]	8-Dec-11	434	1196	9	F	49.3	4.12%	4
23	29-Dec-11	392	866	8	M	<2.0		1
24	13-Jan-12	385	875	11	F	7.1	0.81%	1
25	24-Jan-12	514	2300		F	110	4.78%	4
26	1-Feb-12	447	1130	10	F	20.0	1.77%	2
27	5-Feb-12	547	2529	32	M	<1.0		1
28[2]	1-Mar-12	560		21	M	1.0		1
29	7-Mar-12	431	1197	10	F	11.6	0.97%	2
30	7-Mar-12	388	858	11	F	3.2	0.37%	1
31	8-Mar-12	474	1525	14	F	17.3	1.13%	2

* Reproductive stage: 0. not detectible; 1. immature; 2. mature - not vitellogenic in females or resting in males; 3. well developed vitellogenic oocytes in females, advanced spermatogenesis in males; and 4. hydrated oocytes present in females, and free flowing sperm in tubules and tissue in males.

[1] Caught in lobster pot.

[2] Caught by a recreational fisher.

The gonado-somatic index (GSI) was calculated with the formula: GSI = [Gonad weight (g) / Total fish weight (g)] X 100. No males were sampled that were spermiating (i.e. "running"), although one male (547 mm, 2,529 g) examined on 26 October 2011 expressed milt. GSI of three female fish with hydrated oocytes ranged from 3.11 – 4.78. One of these female fish (434 mm, 1,196 g) was a wild specimen captured on 8 December 2011. A female (514 mm, 2,300 g) that died on 24 January 2012 provided a sample of hydrated oocytes that had been ovulated but not spawned. The diameter of the hydrated oocytes was 850 µm (Figure 1) and the weight of the gonads was 110 g representing a GSI of 4.78.

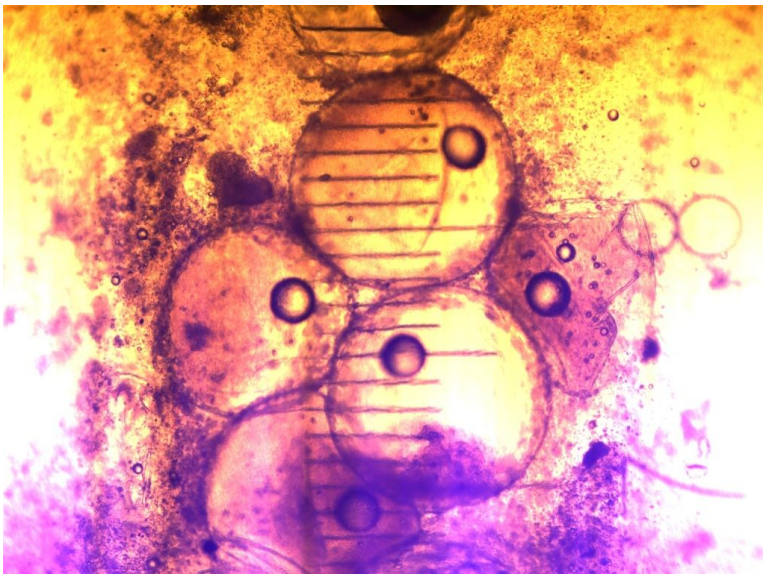

Figure 1. Hydrated and ovulated oocytes (diameter 850 µm) from a harlequin fish that died without releasing oocytes.

These observations indicate that spawning of harlequin fish may occur from late October until the end of January, similar to a number of other marine finfish species in SA waters (e.g. snapper, yellowtail kingfish and mulloway). Comparison of the age of the smallest fish showing reproductive maturation indicates that first maturity for both sexes may be 8-9 years. Both sexes were represented across the range of sizes indicating that harlequin fish are a gonochoristic species (i.e. sexes are separate and do not change during the life of individual fish).

In this study, methods used by Saunders *et al*. (2010) for fish age determination and interpretation were applied. Otoliths (sagittae) were removed from dead fish by cutting with a meat saw horizontally from in front and slightly above the eyes back to the start of the operculum (Figure 2). A second cut was then made from the ventral surface of the head to the end of the first cut that terminated at the operculum. The resulting triangular section was removed to expose the brain. Paired sagitta were removed using fine tipped metal forceps after locating them recessed within the floor of the auditory capsule underlying the brain. A transverse section was prepared from one of the largest pair of otoliths and their opaque zone number was then counted under a dissecting microscope. The fish age in years was then determined to be the number of opaque zones. Ages of harlequin fish sampled in this study ranged from 6

to 32 years (Table 1), providing evidence that the species is long lived. From extrapolation of the data in Figure 3, it appears that it would take at least 2.5 years for wild harlequin fish to grow to a plate size of about 500 g.

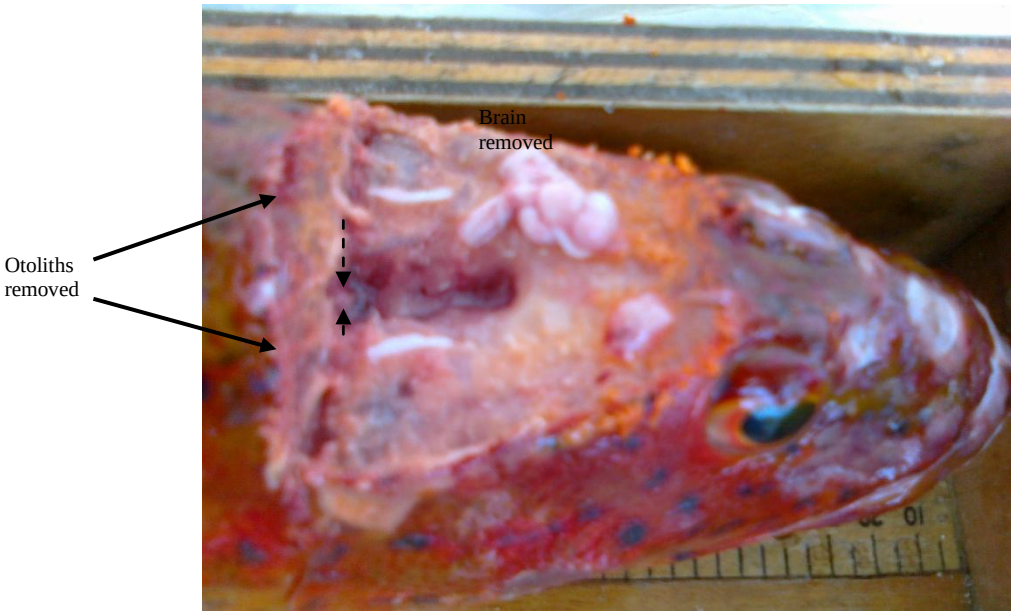

Figure 2. A harlequin fish showing the section of the head removed to extract the otoliths lying within the otic capsule underlying the brain.

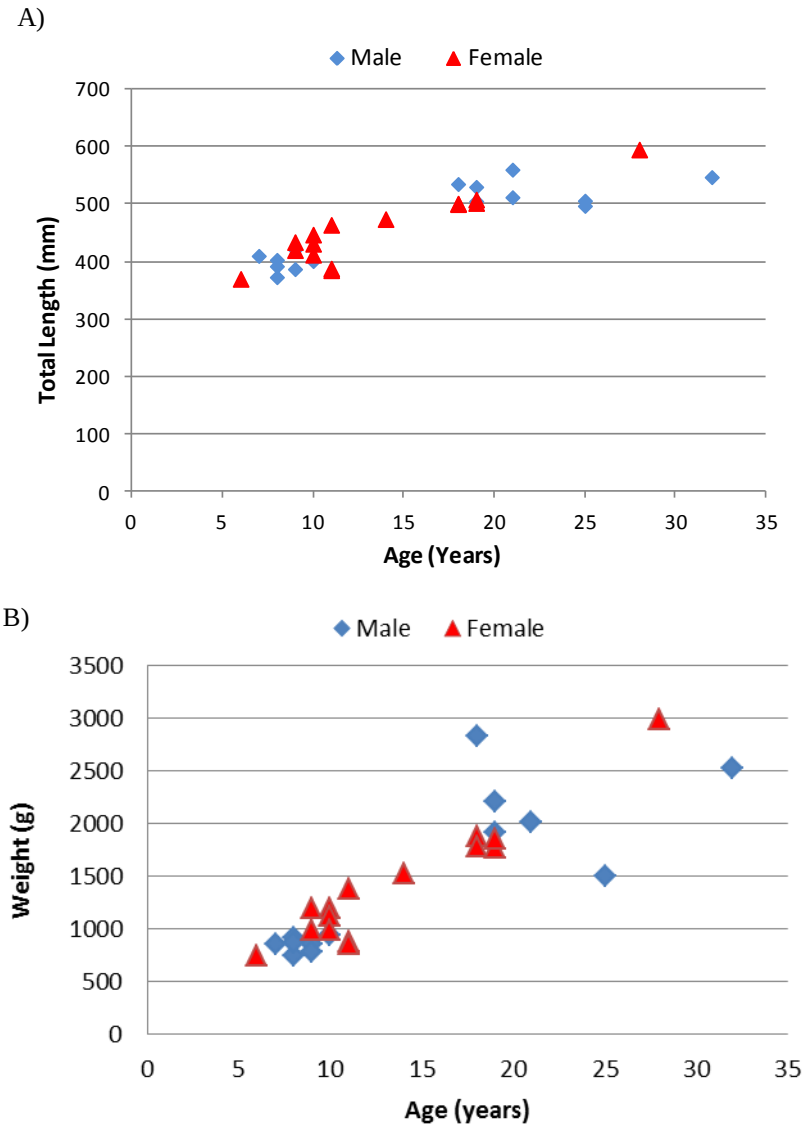

Figure 3. Age and length (A) or weight (B) of male and female harlequin fish, *Othos dentex*, sampled in this study (n = 29).

Mortalities

On 10 April 2011 all 14 fish in one of the two holding tanks died due to a facility seawater system malfunction. Mortalities were also noticed in shelters from early December 2011; pathology reports were inconclusive. Water temperature in the outdoor tanks was 22.5 ^{o}C when these mortalities were first recorded. Subsequently, five fish were transferred to a 2000 L indoor tank supplied with 21 ± 1 ^{o}C flow-through water. Although three fish (60%) in the indoor tank survived the early summer period, all died two weeks after the water temperature increased to 23.6 ^{o}C due to a facility water chilling system malfunction on 21 February 2012.

High water temperature was probably the key factor contributing to harlequin fish mortalities in this study. Ambient temperature of seawater supplied to the captive harlequin fish is likely to be higher than the temperature they would be exposed to where they were captured on the north coast of Kangaroo Island and elsewhere in SA where they occur naturally (Simon Bryars, pers. comm.). Long term exposure to elevated water temperature beyond the tolerance of this species is likely to have adversely impacted reproductive development and ultimately contributed to mortality through chronic stress, adverse physiological effects (i.e. reduced dissolved oxygen, increased metabolism) and greater susceptibility to disease.

Apart from water temperature, it is possible that other factors such as nutritional deficiency or territorial defence may have caused some of the captive harlequin fish mortalities. Trials to introduce other baits/feeds such as prawns and squids to diversify their diet were unsuccessful.

Summary

- Collection of harlequin fish broodstock from the wild should occur over broad areas so that localised depletion of site specific populations does not occur.
- Wild caught harlequin fish can be transferred reasonable distances under appropriate conditions.
- Wild caught harlequin fish can be trained to eat at least one bait fish feed in captivity.
- External copepod parasites can be effectively controlled in captivity.
- The sex ratio of the harlequin fish captured for this study was close to 1:1 and both sexes were represented across the range of sizes, indicating that harlequin fish are a gonochoristic species.
- The highest gonad-somatic index of spawning females was recorded between early December and late January (i.e. summer).
- Ages of harlequin fish from this study ranged from 6 to 32 years.
- Total length and weight of captive harlequin fish were 270 mm to 595 mm and 743 g to 2,996 g, respectively.
- The key likely cause of harlequin fish mortality in this study was higher than typically experienced water temperature (>22 ^{o}C).

Conclusions

Results from this study show that the harlequin fish broodstock population could be established in captivity if the water temperature can be maintained at less than 21 ^{o}C over summer, which is achievable as a finfish hatchery would need a reliable water temperature controlling system to control broodstock sexual maturation. Other techniques, such as fish collection and transportation, parasites control, fish weaning, and daily maintenance have been developed in this study. As harlequin fish are gonochoristic, it would not be needed to manage the year to year unbalanced sex ratios experienced in the hermaphroditic, high value groupers in the same Family Serranidae. The estimated growth rate to the plate size (about 1,000 g) was low; however, this could be offset by the high price assessed by our project client.

To establish a commercial aquaculture entity to farm this species in SA, the next key steps are to investigate: 1) hatchery techniques; 2) grow-out techniques; and 3) cost benefit analyses on business structures. The key impediment would be the uncertainty in obtaining enough wild fish to establish a breeding population and replace losses from mortality.

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