Conservation measures for alternate carp species:

Some of these alternate species are endangered and some are vulnerable to extinction. To conserve these valued carp species, it is important to ensure the following measures:

- Regulation of mesh size to prevent the catching of brooders and young ones during the breeding and larval rearing stages of fish.
- Declaration of sanctuaries in the area where these species are endemic.
- Artificial recruitment may be made to revive the carp species in the areas where these fishes are less available and the catch is declining.
- Prevention of entry of industrial pollutants in the areas where these fish inhabits.
- Public awareness is required to save these fish from extinction.
- Conservation of gametes through gene banking is important for adopting future strategies of replenishment and stock enhancement of these valued carp species.

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Genetic and reproduction technologies for enhanced aquaculture and fisheries management of Murray cod

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Introduction

Fish consumption forms an important aspect in the traditional diet of many communities, particular in Asia. However, increasing consumption of seafood combined with the increasing world population has lead to a substantial rise in demands for aquatic food resources. Today, around three quarters of the world's fish stocks monitored by FAO are fully exploited, overexploited or depleted. The maximum wild capture fishery potential from the world's oceans has probably been reached and there is world-wide over-fishing of inland fishery resources. (FAO 2007). One possibility for alleviating pressure on wild fisheries is through aquaculture (food fish and aquatic plants) which continues to grow rapidly globally (average 8.8% per annum since 1970), more so than for capture fisheries (1.2%) and terrestrial farmed meat production (2.8%) (FAO 2007). Aquaculture is providing an alternative supply pathway by producing both staple fish species and high-value seafood from sustainable production systems and is alleviating pressure on wild fisheries.

In recent decades there has been a revolution in application of genomics and gene-related biotechnology in agriculture and aquaculture (eg. Foresti 2000, Hew and Fletcher 2001, Melamed et al. 2002). Biotechnology research aims to increase production and reduce costs, especially through the manipulation of the genes and chromosomes of cultivated species. Equally biotechnology is also applied to wild fisheries resources to improve their management, for example DNA is being used to differentiate populations and to manage captive breeding programs for stock enhancement/replenishment.

In Australia, through a major project funded by the State Government of Victoria, a biotechnology approach for enhancing aquaculture and fisheries management is being applied to Murray cod (*Maccullochella peelii peelii*) (Percichthyidae) (Ingram et al. 2005b, Rourke et al. 2007a, Ingram 2007). The challenge of this project is to apply advanced genetic and reproduction technologies to this high-value inland species to increase production efficiencies and manage wild populations. With gains in production performance, reliability and profitability, the next five to 10 years could see the expansion of Murray cod farming sector in Australia and internationally. Application of these technologies will also provide extra benefits for the management of native finfish biodiversity, particularly for species such as Murray cod which are declining in the wild and yet continue to be managed for multiple purposes (ie. commercial, recreation, conservation). This article outlines progress made in the project.

Murray cod, Australia's largest indigenous freshwater fish, is an iconic species with significant commercial, recreational, conservation and cultural value (Figure 1). However, over-fishing, habitat loss and modification within the cod's natural range in

Figure1.Large Murray cod angled from the Murray River.

the Murray-Darling Basin (MDB) have seen numbers reduced since the early 1900's. The MDB consist of a network of streams, rivers and water bodies with a catchment area of over million square kilometres, approximately 14% of the land surface of the Australian mainland. Nationally, Murray cod is listed as "Vulnerable" under Australian legislation (Environment Protection and Biodiversity Conservation Act 1999), which is designed to protect its conservation status. Since the 1980's, over 11 million hatchery-reared juveniles have been released into the wild to enhance recreational fisheries and for conservation purposes. Murray cod has excellent aquaculture prospects and small quantities are already being sold into domestic and international markets (Ingram et al. 2005a, Ingram and De Silva 2004). Some aquaculture attributes of Murray cod include, established hatchery production of fry, readily accepts artificial food, hardiness to physical handling, fast growth rates, can tolerate high stocking densities and has a high demand within the Asian community.

Project aims

The project aims to develop and apply genetic and reproduction technologies to boost the profitability and sustainability of the newly emerging Murray cod aquaculture industry in Australia. The selective breeding of Murray cod will involve using genetic markers (microsatellites) to identify broodfish that possess favourable production traits of commercial importance (ie. rapid growth, robustness, disease resistance, skin colour, fat content etc.). These broodfish will then be used to breed new elite strains of Murray cod suited to aquaculture.

The project will also investigate the use of controlled reproduction technologies, (ie. out-of-season spawning, chromosome-set manipulation, hybridisation and cryopreservation) to improve stock management and to enhance the performance and value of selectively bred strains of Murray cod. These technologies will allow production of sterile seedstock that will provide for biosecurity and protection of intellectual property (IP) invested in selective breeding.

Microsatellite markers and genetic tools developed for the selective breeding program will also be used to study the genetic diversity of wild Murray cod populations across the MDB. This work will assist in the protection and management of these populations, and will be used to develop genetically sound stock enhancement practices to support recreational fishing.

The project is being undertaken by scientists at the Department of Primary Industries (DPI), Victoria, Australia at the Attwood and Snobs Creek centres and forms part of the Our Rural Landscape (ORL) Initiative program. The Attwood group is conducting animal genetics and genomics research for the dairy, beef, sheep and abalone industries. It has built up an impressive collection of genetic material, as well as expertise in molecular genetics and bioinformatics. The Snobs Creek group has a long history of fisheries and aquaculture research, and has played a key role in the development of captive breeding and production techniques for Murray cod and other native fish species.

Developing biotechnology capabilities by government and industry will provide clear triple bottom line benefits which are;

1. Economic; through the development of the Murray cod aquaculture industry and sustaining Murray cod recreational fisheries;
2. Environmental; from using microsatellite markers to help understand biodiversity in Murray cod populations, and;
3. Social; through enhanced genetic management of wild populations for conservation and recreation.

Project progress

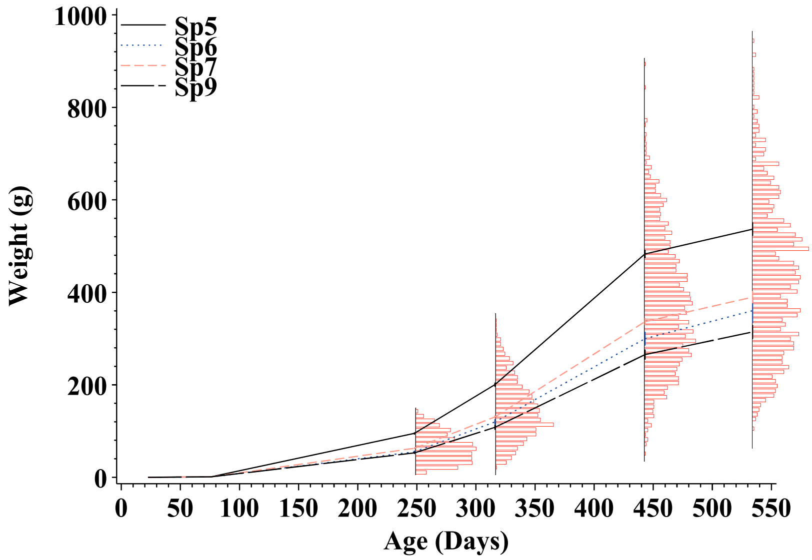
Selective breeding

Currently, approximately 4,600 fish from 40 genetically diverse families (1st generation stock) of Murray cod, obtained from two government hatcheries and three private hatcheries over two different breeding seasons, have been established in custom built recirculating aquaculture systems to create the founder population. An additional 20 families will be obtained during the current breeding season to boost diversity of the founder population. These fish, once mature will provide a genepool from which new broodstock possessing traits of interest will be used to breed new domesticated strains of Murray cod selected for high performance (growth etc.) in aquaculture. Some fish are now reaching maturity and will be ready to spawn for the first time in 2008.

An industry survey of fish farmers and fish retailers indicated that important traits of interest for the aquaculture of Murray cod included fast growth, hardiness (eg. stress resistance and disease resistance) and survival during production, while at market, fish size, skin colour and flesh texture (amongst others) were important. Retailers prefer larger fish (>2 kg), and lighter coloured fish, typical of those caught from the wild, over darker coloured fish. This information will be used to guide the selective breeding program for Murray cod.

In order to link the microsatellite markers to the aquaculture traits of interest, fish from four families of known parentage were combined at three weeks of age and communally-reared. At eight months of age these fish were implanted with microchips (Trovan Pty Ltd, Australia) to identify individuals, and a tissue sample was collected from each fish for DNA analysis. Subsequent measurement of these fish showed that a number of important traits were quite variable within and between families. Fish from one family in particular (Sp5) grew faster (Figure 2) and more survived than in the other three families. At 14 months of age, weight ranged from 57g to 886g. Researchers also observed considerable differences in fish condition and skin colour (Figure 3).

Figure 2. Growth of four families of Murray cod reared communally (lines = mean values), and frequency distribution of weight at four sampling dates (bars).



We have successfully amplified 103 microsatellites in Murray cod, of which 101 were polymorphic and two were monomorphic (Rourke et al. 2007b). Excluding the monomorphic loci, the number of alleles per locus ranged from two to 19 alleles per locus (mean seven alleles per locus). The expected heterozygosities ranged from 0.066 to 0.95 (mean 0.64), and most loci were in Hardy-Weinberg equilibrium. These new loci have been used for the identification of quantitative trait loci to improve the productivity of cultured Murray cod, and to assess wild and hatchery stock structure of Murray cod for management purposes. A genetic map for Murray cod is currently being developed and analysed for the four communally reared Murray cod families. So far, 20 linkage groups, with between two and seven markers per group, have been identified. Cross-amplification of the loci developed for Murray cod was tested on 19 other species of fish. Within the Percichthyidae, 79-94% of loci crossed-amplified in three other *Maccullochella* species and 18-38% four *Macquaria* species (Rourke et al. 2007). These results make this set of microsatellite markers an extremely useful resource for future genetic studies of percichthyids.

A subset of microsatellite markers have been successfully linked to a number of important aquaculture traits, including growth (weight at measurement and specific growth rate), skin colour, fish condition and carcass fat content (Figure 1). However, analyses performed on the surviving fish found no evidence of any of the DNA markers being inherited with the “survival” trait. Nevertheless, this information can now be used to select broodstock with these traits to produce new, high performing strains of fish for the aquaculture industry.

Reproduction

Research into the controlled reproduction of Murray cod is being conducted on a group of mature broodfish held in a recirculating aquaculture system under an artificial photo-thermal regime. These fish were successfully induced to spawn outside their normal breeding season by manipulating the temperature and light regimes in the recirculating aquaculture system and hormone-induction of ovulation (Figure 4). In 2004, when the first spawning trials were conducted, 90% of fish injected with hormone (HCG) ovulated and were stripped, but hatch rates averaged 3%, and were substantially lower than hatch rates observed in natural pond spawnings at DPI, Snobs Creek (mean 50%). However by the third year of trials hatch rates of up to 67% were obtained. Controlled breeding of this species will increase the flexibility of the selective breeding program and enable greater control over fish mating and seedstock production.

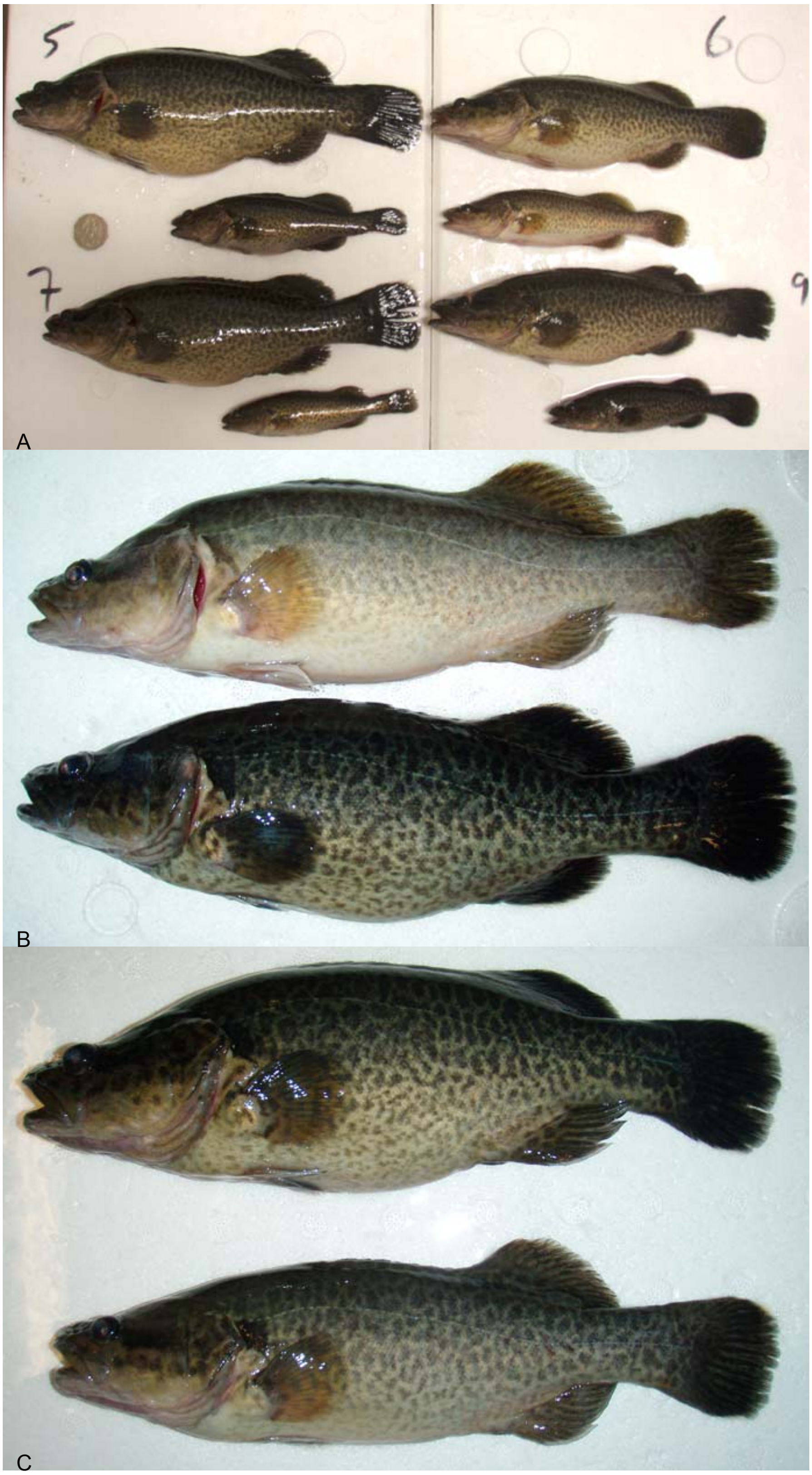
Triploid fish (fish with three sets of chromosomes) are desirable in aquaculture because they are generally sterile. Since no energy is spent in the development of gonads, these fish exhibit faster muscle growth. Triploidy is also used as a means of genetic containment by reduction of unwanted reproduction. Triploidy was induced in Murray cod by shocking eggs shortly after fertilisation. Eggs in some shock treatments failed to hatch while in other treatments hatch rates ranged from <1% to >100% of control (non-shocked) hatch rates. Heat and cold shocks failed to induce triploidy, whereas some pressure shocks induced various levels of triploidy (up to 100% in some replicates) (Figure 5). Further experiments are now being conducted to refine techniques.

Hybrid fish are used in aquaculture to increase growth (hybrid vigour), transfer or combine desirable traits between species and reduce unwanted reproduction through production of sterile offspring. Hybridisation was successfully induced in both direct and reciprocal crosses between Murray cod and trout cod (*Maccullochella macquariensis*). Murray cod x trout cod hybrids, though rare, occur naturally in the wild where both species occur sympatrically. A small number of hybrids are currently being reared to evaluate their aquaculture performance (eg. growth and environmental tolerance) and ultimately to determine their fertility.

In collaboration with the Monash Institute of Medical Research (Melbourne), we are investigating options for cryopreserving sperm in liquid nitrogen as a means of cost-effectively storing genetic material for conservation and selective breeding programs (Figure 6). Trials have identified cryoprotectant composition and freezing methods for Murray cod sperm that provide high levels of sperm motility post-thawing. In fertilisation trials, we have achieved hatch rates up of to 65% of the hatch rates for eggs fertilised with fresh sperm.

Genetic analyses of captive Murray cod broodstock and their offspring have provided insights into the previously unknown breeding habits of this species. Murray cod are generally believed to form monogamous pairs and breed once a year. In captivity, broodstock are allowed to spawn naturally in ponds and during the breeding season nesting boxes located

Figure 3. Variation communally reared Murray cod in size. (A) growth variation within and between four families. (B) Skin colour. (C) Condition (fattiness).



in the broodstock ponds are regularly checked for spawnings. However, parentage analysis of captive-spawned offspring has revealed that broodstock are occasionally polygynous with up to 17% of spawnings involving three parents (mostly 1 male and 2 females). Some fish (both male and female) spawned twice in a single season, and larger males contributed more to spawnings than smaller males. These results have substantial implications for genetic management of captive broodstock used in stock enhancement programs.

Early detection of sex is important in evaluating reproduction technologies that affect or manipulate sex. However, outside the breeding season Murray cod cannot be accurately sexed without applying invasive techniques whilst juveniles cannot be sexed at all. We found that testing for the presence of vitellogenin (yolk protein) in blood plasma, was a reliable method for non-destructively identifying maturing and mature female Murray cod.

Population genetics

In order to determine the genetic structure of contemporary wild Murray cod, tissue samples (finclips and fish scales) were obtained from 700 fish from 19 river catchments across MDB. A historical collection of Murray cod scales of 361 fish collected from six southern river catchments between 1948 and 1953 were compared against contemporary samples (post 1995) to identify temporal changes in the genetic structure of Murray cod in the southern part of the MDB, and to identify potential impacts of stock enhancement programs, which commenced in the early 1980's.

Genetic analysis of contemporary fish samples revealed up six populations across the MDB. Fish from two river catchments (Lachlan and Macquarie-Bogan) each represent a discrete genetic cluster, while fish from three others (Border, Namoi and Gwydir) show a slightly lower degree of differentiation and evidence of restricted gene flow. Fish from other MDB catchments are apparently one large panmictic population (Figure 7). These results indicate that the populations in rivers flowing into the Murray River are highly connected, while those terminating in swamps and wetlands, (Lachlan and Macquarie-Bogan), are historically isolated.

Results of this study suggest that stock enhancement practices have had a mixed effect on the Murray cod populations in the MDB. Temporal comparisons between the historical and contemporary samples from five southern catchments showed limited genetic differentiation, indicating no change in either genetic diversity or structure in these catchments over the past half century, despite the commencement of stock enhancement in the 1980's. This is probably due to broodstock being obtained primarily within these southern catchments, which represent a single panmictic population and continual replacement of broodstock in breeding programs with fresh stock from the wild. On the other hand, there is evidence that stocked fish have interbred with local populations in two catchments (Macquarie-Bogan and Gwydir), while one distinctive population (Lachlan) has retained its genetic uniqueness despite extensive stocking of fish into large impoundments within these catchments. These manmade barriers may have prevented stocked fish from mixing with native strains in the rivers downstream.

This is important information for the long-term management of the genetic diversity of wild populations of Murray cod. The data has shown that some populations are unique and need to be managed accordingly. We are now using these data to develop a model to evaluate the effects of different breeding and stocking practices on the genetic diversity of wild populations. This model will assist fisheries managers to implement genetically sound stock enhancement programs.

Conclusions and future work

Murray cod aquaculture is a new and developing industry that exhibits efficient and profitable use of natural resources (water and fish). This project has established an excellent foundation for establishing a selective breeding program for the species (family lines and tools for selection). While no selec-

Figure 4. Hand-stripping Murray cod eggs.

Figure 5. Application of hydrostatic pressure to induce triploidy in Murray cod eggs.

Figure 6. Straws of Murray cod sperm being frozen in liquid nitrogen.

tive breeding took place in the current project, due to the limited timeframe, the results from this work are the first stage of such a program. Now that a genetically diverse founder population of broodstock has been established and microsatellite markers have been linked to favourable aquaculture traits, a “proof-of-concept” project will be undertaken to evaluate the performance of selectively bred strains of Murray cod. In addition, discussions with potential industry partners to commercialise the breeding program have already commenced.

Information from this project will assist fisheries managers in assuring the future of wild stocks, ensuring sustainability of aquatic resources and maintenance of biodiversity. The existence of unique populations of Murray cod and are now being recognised and being incorporated into management plans for the species by some fisheries agencies. The microsatellite markers developed for Murray cod are now being used by other researchers in population genetics studies of percichthyid species for conservation purposes.

Another achievement of this project has been the development of new capabilities in fisheries and aquaculture genetics and genomics within the DPI. These capabilities will be applied to other aquatic species for aquaculture (eg. selective breeding of blue mussels, *Mytilus* spp.), sustainable fisheries management (development of genetically sound stock enhancement practices for native species) and aquatic species conservation (eg. genetic structure of stocked populations of the endangered trout cod).

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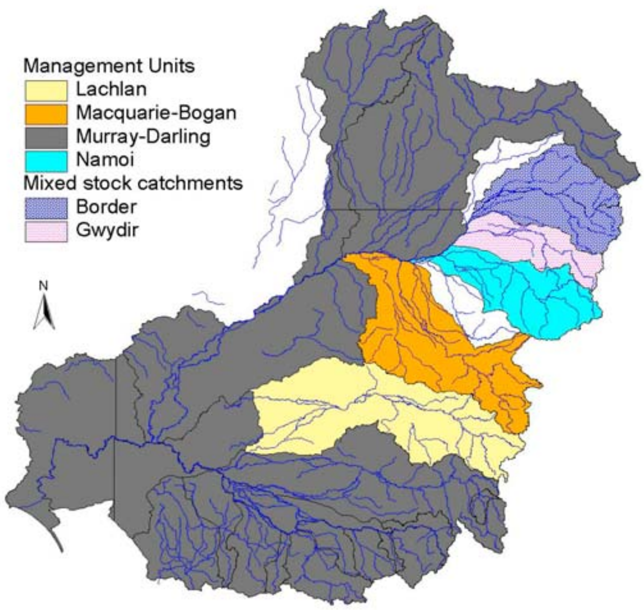
Figure 7. Potentially unique populations (management units) of Murray cod in the Murray-Darling basin.



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Effluent and disease management in traditional practices of shrimp farming: A case study on the west coast of Sabah, Malaysia

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Introduction

Similar to other aquaculture activities, the production process of shrimp farming is not without environmental implications. To a global context, the adverse environmental impact of unplanned and uncontrolled expansion of shrimp farming has prompted widespread criticisms (Naylor et al. 1998). Disease problems and environmental issues in shrimp farming have caused worries about the sustainability of traditional farming practices (Otoshi et al., 2005). Persistent disease and environmental threats originating from traditional practices have also caused concern in Sabah. A thorough assessment of the farming practices will provide a basis for recommendations for the most viable way of producing shrimp consistent with sustainability criteria. This has been attempted in the present study.

Materials and methods

In the process of data collection, this case study used method based on social science approach - technical site observations and interviews. Social science research offered a way of better understanding people who were engaged in the fisheries activities and the social dimensions needed for inclusion in the management process (Kaplan and McCay, 2004).

The farms were located on the west coast of Sabah, in Tuaran, Kota Belud, Kudat, Kuala Penyu and Beaufort. Three field visits were made to each of the shrimp farms during August-December 2005. This was followed by two more visits in August 2006 for reliability checks and to validate observations made earlier. These farms were chosen because of their willingness to cooperate unconditionally. The samples in this study were farm managers and farm technicians, working directly at the farms. Semi-structured interviews were performed on site, face to face, using a printed form as guidance. Questions were asked about farm management practices for disease and effluent management.

The observations were obtrusive; the farm managers were informed in advanced that site observation and interview would take place. Observation focused on obtaining direct and generally indisputable verification of obvious behaviour which can also be used to measure subjective experiences such as attitudes (Singleton and Straits, 2005). The Department of Fisheries, Sabah record showed as of August 2005 that there were 21 active shrimp farms on the west coast of Sabah. Ten shrimp farms were included and taken as purposive and convenient samples, representing about 50% of the total shrimp farms in that area.

The aim in a qualitative research is to describe the process and events involved rather than their distribution and therefore, sampling is purposive and does not attempt to be statistically representative (Rice and Ezzy, 2001). Questions and observations were designed so as to elicit this information. In such studies it is a general distribution of processes that is considered necessary.

Even though the shrimp farms are accessible by road, most of them are scattered and it took between two to three hours to reach some of the farms. In such a situation, this convenience or purposive sampling could be appropriate (Singleton and Straits, 2005). Due to limited time and resources, the number of shrimp farms included in this study was considered sufficient to achieve the objective of the study. The decision to select study sites was based on: 1) availability of funding, 2) allocation of time, 3) willingness of participants, and 4) accessibility.

Results and discussion

Results of this study are presented in a qualitative manner. The observed variables and questions asked are discussed to explore the qualitative dimension of traditional practices. This case study revealed that the nature of its operation is purely for commercial purposes. The size of shrimp farms varied from 7 acres to 100 aces.