THE FEASIBILITY OF STOCK ENHANCEMENT AS A MANAGEMENT TOOL FOR DUSKY KOB (*ARGYROSOMUS JAPONICUS*) IN SOUTH AFRICA

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ABSTRACT

The dusky kob, *Argyrosomus japonicus*, is a popular South African "line fish" whose stocks have dwindled to dangerously low levels of between 1% and 4.5% of pristine spawner biomass per recruit. *A. japonicus* stocks are currently managed by means of minimum size restrictions and daily bag limits, and as a result of the inability of these measures to facilitate the recovery of the species over a realistic time frame, the need for an alternative management plan has become apparent. Given the status of the stock and management regime, stock enhancement appears to be an appropriate option to be investigated.

This study evaluates the feasibility of stock enhancement as a possible management tool to assist with the recovery of this important South African linefish species. By evaluating the genetic and ecological implications related to stock enhancement, identifying a suitable tagging method for post-release monitoring, and evaluating the economic feasibility of such a programme, any fatal flaws would become immediately apparent. This coupled with the required framework for the development of a management plan for stock enhancement of *A. japonicus* provides direction further research and actions required in order to utilise stock enhancement as a management tool.

Due to the nature of stock enhancement, there are several ecological and genetic issues that arise from such a programme. These issues were reviewed and the issues that were relevant to stock enhancement of *A. japonicus* identified. Ecological concerns that arose

included those of competition, disease and seed quality, while genetic issues were concerned mainly in the possible loss of genetic variability and consequent reduction in fitness of the stock. Fortunately the technology exists to evaluate the effects and likelihood of these problems occurring as well as to minimise the likelihood of them occurring. By taking a scientific approach to stock enhancement, hatchery management, and release strategies can be manipulated in such a way as to minimise any negative effects that may be caused. Both ecological and genetic effects of stocking indicate a need for post-release monitoring of stock enhancement programmes.

Stock enhancement requires a post-release monitoring programme, which in turn relies on an ability to distinguish between hatchery reared and wild fish. A study was conducted to evaluate the suitability of coded wire tags (CWT), visual implant fluorescent elastomers (VIFE), and oxytetracycline (OTC) as a means of distinguishing between hatchery reared and wild *A. japonicus*, for the purpose of a post-release monitoring programme. OTC appeared to be the most suitable as it produced 100% retention over a five month period compared to 62% and 61% for VIFE and CWT respectively. OTC is therefore suggested as a tagging method for the purpose of post-release monitoring of the stock enhancement of *A. japonicus*.

To evaluate a possible funding option for stock enhancement of *A. japonicus* in South Africa, a willingness-to-pay survey, based on a “user pays” approach using recreational fishing permits as a vehicle for payment, included 102 recreational anglers in the Plettenberg Bay area. The survey showed that generally anglers were willing to pay more than the current amount for the recreational fishing permit. This promising result, coupled with the fact that there are approximately 450 000 recreational anglers leads to the belief that there is potential for a substantial increase in the funds generated for the Marine Living Resources Fund through recreational anglers. Stock enhancement should not be ruled out on the basis of economic feasibility yet as there is potential for it to be sustained by the users of the resource.

An *A. japonicus* juvenile production costing model was created taking into account setup and running costs of a hatchery for *A. japonicus*, based on known parameters from existing facilities, and adjusting them to meet the requirements of a stock enhancement facility. Estimates varied according to the number and size of fish for release (values which can only be decided upon after further research), with setup estimated to be between R 10 000 000 and R 30 000 000 and annual running costs between R 2 400 000 and R 6 700 000 annually. These figures were dependant on the size and number of fish being produced, with production ranging between 100 000 and 5 000 000 fish of between 50 mm and 150 mm, and a broodstock of 150 individuals.

Given the need for alternative management of *A. japonicus* in South Africa and the lack of evidence to suggest that it is an unfeasible option, this project has found no reason why further investigation into the use of stock enhancement for the management of *A. japonicus* should not proceed further provided the fishery is shown to be recruit limited. The technology and ability to overcome possible ecological and genetic problems exists, a suitable means of tagging for post-release monitoring exists, as does a realistic funding option. There is a substantial amount of research that must be done prior to stocking, for which a base framework is provided.

CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION TO STOCK ENHANCEMENT OF *ARGYROSOMUS JAPONICUS* IN SOUTH AFRICA

1.1. Introduction

The Dusky kob, *Argyrosomus japonicus* (Temminck & Schlegel, 1843) is a popular linefish species among commercial and recreational anglers in South Africa. However, due to fishing pressure, inadequate management (Sauer *et al*. 2003), and its life history characteristics, there has been a steady decline in *A. japonicus* landings over the past 20 years, and Griffiths (1997a) concluded that the species was severely overfished. It is thus appropriate that the existing management strategy is re-evaluated, and that alternative management tools are considered that may facilitate the recovery of the species.

Stock enhancement (stocking cultured organisms to replenish or increase the abundance of wild stocks) is a management tool which has been applied to many species around the world. However, stock enhancement has been criticised as a management tool because programmes have often been politically rather than scientifically motivated, resulting in ineffective stocking strategies and unquantified outcomes (Leber 2004). This has led to the evaluation of previous successes and failures and the adoption of a "responsible approach" to stock enhancement (Blankenship and Leber 1995), whereby decisions are based on sound scientific information. If the assessment of the feasibility and management decisions for stock enhancement is conducted in this manner, then stock enhancement has the potential to be a very effective management tool; one which may be used to facilitate the recovery of *A. japonicus* in South Africa.

This project investigates the feasibility of using stock enhancement as management tool for *Argyrosomus japonicus* in South Africa, and addresses some of the main concerns of managers, regarding its use. The first step towards creating a framework on which to set goals and base management decisions is to understand the species in question, and to evaluate the need (if any) for stock enhancement.

1.1.2. Biology and Life History of *Argyrosomus Japonicus*

Argyrosomus japonicus is a large Sciaenid that reaches a maximum size of about 1.8 m and 75 kg (Griffiths 1997a). It occurs between Cape Agulhas and northern KwaZulu-Natal in South Africa (Figure 1.1), along the entire south coast of Australia, and from Hong Kong to North Korea and Japan (Griffiths 1996). *A. japonicus* is prevalent in estuaries, and inshore to a depth of approximately 100 m, depending on its life history stage (Griffiths 1997a). Maturity is attained at approximately 920 mm (five years) and 1070 mm (six years) by males and females respectively.

Figure 1.1: Distribution map of *A. japonicus* in South African waters.

In South Africa a large proportion of the adult *A. japonicus* undergo an annual northward spawning migration up the east coast to KwaZulu-Natal between August and October (Griffiths 1997a). Spawning continues from October to January in the Southern and South Eastern Cape when the adult fish return from KwaZulu-Natal (Griffiths 1997a). Some adult fish, however, do not undergo this migration, but remain in the South Eastern Cape and spawn there between October and January. Warmer water at this time of the year is thought to act as a cue for the fish to start spawning (Griffiths 1997a). Eggs and larvae are dispersed passively along the South African coastline by the Agulhas current. Spawning coincides with the predominantly rainy season in the majority of South Africa when freshwater influence is greatest in the estuaries. This freshwater provides olfactory cues to the early juveniles, of between 20 mm and 30 mm, which recruit into the estuaries, using them as nursery areas (Griffiths 1996, Griffiths 1997a). Due to the freshwater influence on these rivers, they are usually turbid, suiting *A. japonicus*, which show preference to more turbid estuaries (Whitfield 1998) such as the Breede and Great Fish Rivers. These young juveniles remain in the upper reaches of the estuaries in salinities of between 0 ‰ and 5 ‰ where there are fewer predators (Griffiths 1997b). As they grow and become less vulnerable to predation, their distribution expands to include the middle and lower reaches of the estuary where they have been shown to feed mainly on mysids and small fish (Griffiths 1997b). Once the juveniles reach a total length (TL) of approximately 150mm they venture out of the estuaries into the surf zone and remain in the near shore region until maturation (Griffiths 1997a), when they move offshore into deeper water (Griffiths 1996), they do, however, frequent estuaries and the surf zone throughout the duration of their lives

Tagging studies by Griffiths (1998) have shown that juvenile *A. japonicus* are not highly migratory, with 83% of the 253 recaptured fish being caught within 10 km of the release site, despite periods of liberty of up to 1713 days. This means that while the juvenile population is localised to specific areas a large proportion of the adult population of *A. japonicus* form part of a national stock. However, as is discussed in Chapter 2, further genetic studies are required to provide more detailed information on the life history strategies and population structure of *A. japonicus*.

An important study which needs to be done for *A. japonicus*, is that of determining the size and shape of the stock in South African waters. It is important to have a good understanding of these aspects when developing a management protocol, especially one for a species which appears to be in trouble and requires stocks to be rebuilt to sustainable levels. This includes getting an idea of the population size and age structure. From this, managers will be able to identify where exactly the problems causing the reduced spawner biomass per recruit lie, and a management protocol developed whereby the cause of the problem is eliminated. This information will also be extremely useful in determining the suitability of various management tools.

1.2. Evaluation of the management of *Argyrosomus japonicus* in South Africa

Considering the apparent decline in *A. japonicus* stocks under the current management regime, scientists need to evaluate the current management techniques critically. If it is found that traditional management tools are unlikely to facilitate the recovery of *A. japonicus* in South African waters then the use of other management tools may be necessary and should be considered. In order to contextualise stock enhancement as a management option for *A. japonicus*, the current management framework is reviewed. It is generally accepted that stock enhancement comes with certain risks, and that it is should only be considered when conventional management options have been proven inadequate (Bell 2004).

The South African linefishery is made up of a commercial and a recreational sector. The commercial linefishery employs in excess of 4100 individuals (Sauer *et al*. 2003b) and generates over R 64 000 000 annually (Sauer *et al*. 2003a), while the recreational sector is comprised of approximately 450 000 permit holders annually. Because *A. japonicus* is a linefish species, it is this sector of the fishery that makes up the majority of *A. japonicus* catchers. *A. japonicus* has traditionally been one of the main target species of the South African linefishery, with the species making up over 6% of the total commercial linefish catches in 1985, however, today they make up less than 1% of the total linefish catch (data obtained from Marine and Coastal Management). Commercial line fishing takes place offshore, and, therefore, targets mature *A. japonicus*, while recreational angling takes place mainly from the shore and in estuaries, contributing a large proportion of juvenile catches.

Currently, the linefishery in South Africa is managed under the South African Marine Living Resources Act (Act No. 18 of 1998); the goals of which are:

- To actively facilitate the recovery of over-exploited line fish stocks, and to maintain such stocks at optimum levels of production
- To ensure user participation in the development and implementation of management measures
- To ensure that the process of granting access to linefish resources is fair and equitable

These goals are well aligned with the overall objective for the management of the South African line fishery, which is "to manage the linefish resource of South Africa so as to ensure the equitable sustainable utilization of these resources." (Griffiths *et al.* 2000).

The current management protocol in place for *A. japonicus* does not achieve the first of these goals, which is to facilitate recovery of over-exploited linefish stocks. The management protocol must therefore be reassessed in order to facilitate the recovery of these fish so that a sustainable fishery may be created.

The management of *A. japonicus* has undergone several changes over the years. Until 1993, the two species *Argyrosomus japonicus* and *Argyrosomus inodorus* where thought to have been a single species, *Argyrosomus hololepidotus*. This had major implications for the management of *A. japonicus,* as the two differ from one another in terms of life history strategy, with *A. inodorus* reaching maturity at 340 mm and *A. japonicus* at approximately 1000 mm (Griffiths and Hecht 1993). As the minimum legal size is commonly set at the approximate size at which maturity is reached, giving fish the opportunity to reproduce before entering the fishery, the size limit for "*A. hololepidotus*" was set at 400 mm. The misclassification of the two species and subsequent poor management of *A. japonicus* led to recruitment overfishing in *A. japonicus*, as juveniles were included in the fishery and were not given a chance to reach maturity and reproduce before being caught. Although "*A. hololepidotus*" was recognised to be two species in 1993, *A. inodorus* and *A. japonicus* were still managed as a single species until 2004, when the size limits and daily bag limits for *A. japonicus* were changed to those shown in Table 1.1.

Table 1.1: Daily bag and Size limits for *A. japonicus* for recreational fishers (Anon. 2004).

Region / area	Minimum size	Daily bag limit	Restrictions
Cape Agulhas – Umtamvuna River (from a boat at sea)	50 cm	5	1 fish > 110 cm
KwaZulu-Natal (from a boat at sea)	40 cm	5	1 fish > 110 cm
Whole of South Africa (estuaries and shore)	60 cm	1	

Daily bag limits are based on catch records which provide an indication of the fraction of catch that would be protected from being caught by a certain bag limit (Attwood and Bennett 1995). Until 1992 there was no daily bag limit for *A. japonicus* and anglers were allowed to keep all legal size (>400 mm) *A. japonicus* caught. In 1992 the daily bag limit was set at 10 fish per fisher (Attwood and Bennett 1995). This however was shown to be ineffective as it only caused an estimated reduction in fishing mortality of 0.1%, as very few fishers attained the daily bag limit (Attwood and Bennett 1995). In 2000 the South African linefishery was declared to be in a state of crisis (Sauer *et al*. 2003b) and as a direct response to this regulations were changed in 2004. The size limits and daily bag limits for *A. japonicus* were changed to those shown in Table 1.1.

Had the daily bag limit been set at one fish per fisher in 1995, as it is today, it is projected that the reduction in fishing mortality would have been an estimated 46.1% (Attwood and Bennett 1995), however, as the stocks have since diminished further, the daily bag limit of one fish per angler is less effective.

Enforcement of, and compliance with these regulations plays a vital role in their effectiveness. This is problematic in the Southern Cape where only 29% of the commercial fishers respect minimum size limits, and both recreational and commercial vessels are inspected at an average of only once in a 12 month period (Sauer *et al.* 1997). Although further studies are required into the size and structure of *A. japonicus* stocks in South African waters, studies have shown *A. japonicus* to have been so heavily exploited in the past that the spawner biomass per recruit of the species is estimated to be between 1% and 4.5% of pristine (Griffiths 1997), and it is possible that it may have decreased even further since. To put this into perspective, it has been demonstrated repeatedly that there is a high risk of collapse of a species when spawner biomass per recruit is reduced to below 20% to 30% of pristine, which is well above the current level. This demonstrates the inability of the current management measures to sustain the *A. japonicus* stocks in South African waters, and more so to facilitate the recovery of the stocks.

Because *A. japonicus* stocks have been so heavily depleted, current management tools do not appear to be sufficient to facilitate their recovery. Griffiths (1997a) calculated in 1997, that with size and daily bag limits set at what they are today, and provided the regulations are followed, it would take over 40 years for *A. japonicus* stock to recover. However, with further decline in the stocks since 1997, it is reasonable to assume that the subsequent alteration in size and bag limit would be even less effective than if it had been implemented in 1997, as opposed to the end of 2004, thus taking well over 40 years for stocks to recover. This is an unreasonably long time, and with the widespread non-compliance in South Africa it seems unlikely that these traditional management tools will facilitate the recovery of *A. japonicus*. Intervention is therefore required, in the form of alternate management measures, for the *A. japonicus* stocks to be increased to an acceptable, safe level.

It is important to distinguish whether the problem lies in the effectiveness of the management tool in place or with the application thereof. Theoretically, according to Griffiths (1997a), the current management should prevent further decrease in *A. japonicus* stocks and, over a long period of time (> 40 years), facilitate the recovery of the stock. The time period of the predicted recovery would, however, be too long. The main problem lies with the application of the management. Regulations are not effectively enforced and compliance is poor, resulting in the management measures being ineffective. This combination of a management plan that can sustain but not facilitate recovery of the stock in a reasonable time frame and lack of implementation thereof, requires intervention in the form of improved compliance coupled with a means to facilitate recovery of the stock quickly.

One option to attempt to facilitate the rapid recovery of *A. japonicus* stocks would be to implement a total ban on the capture of the species (reduce the daily bag limit from one to zero). Due to the popularity of *A. japonicus* as an angling species this would, apart from leading to an uproar among anglers, lead to a decrease in the number of anglers buying recreational permits, which would result in a decrease in the money being generated for the management and enforcement of regulations on the linefishery. This is probably not a wise option as a large number of other species also rely on this for their management.

Where traditional management tools have failed, stock enhancement can often be used as an alternative or complementary tool. Bell (2004) suggests that when unduly long time periods are predicted for replenishment using other management measures, stock enhancement should be used. This is indeed appears to be the case with *A. japonicus* as highlighted above. However, Bell (2004) also emphasizes the importance of compliance with traditional management tools for the management of an enhanced fishery. It is, therefore, important that that stock enhancement be used in conjunction with traditional management tools.

A suite of management measures including traditional and alterative tools are required to facilitate the recovery of *A. japonicus* in South Africa. This means that the current management needs to be reviewed and altered accordingly, for example increasing the size limit. There is also a need for the investigation into the traditional tools that are not currently in place, such as a closed season or a total ban on *A. japonicus* catches. A ban on commercial landings of *Pomatomus saltatrix* in KwaZulu Natal in the 1980's was effective in the recovery the species (van der Elst *et al*. 2000), and a similar ban may prove to be effective for that of *A. japonicus*. It should also be investigated, whether the traditional options can be improved enough to facilitate the recovery of the stock on their own, or whether alternative measures are required. A major contributing factor in the effectiveness of any management tool is the level of compliance with regulations that may be in place. Improved compliance with traditional management should be high on the priority list of fisheries managers. However, before a combined traditional/alternative management approach, using stock enhancement, can be taken, certain issues need to be evaluated to determine whether it is technically feasible, economically viable, and whether there are unacceptable risks involved. Furthermore, stock enhancement will only be considered, in South Africa, if the collapsed stock is shown to be recruit limited (DEAT 2006).

1.3. Stock enhancement

In order to contextualise the use of stock enhancement for the management of *A. japonicus*, an understanding of the principles of stock enhancement is required (Leber 2004). A brief review of stock enhancement thus provided below.

Stock enhancement of marine fishes involves the spawning and rearing of larval fish to their juvenile stage, under controlled conditions, and the release of these juveniles into the wild in order to augment depleted natural populations (Serafy *et al*. 1999) (Figure 1.2) usually for the benefit of the public as a whole, rather than just a single user group (Cowx 1999, Howell 1998). This method of augmenting heavily fished stocks is becoming increasingly popular (Bert *et al*. 2003, Leber *et al.* 2004). Stock enhancement is appealing due to the simplicity of the concept. The naturally high mortality, commonly the case among juvenile fishes, is compensated for by growing them in a hatchery to a size at which this mortality is greatly reduced (Ye *et al*. 2005).

Concern about the depletion of wild fish stocks and plans to use stock enhancement as a management tool were reported as long ago as 1867, with the hatchery rearing and release of *Alosa sapidissima* (shad), in New England, into the wild (Liao *et al.* 2003). Subsequently hatcheries were established in Canada, France, Great Britain, New Zealand, and Norway, for the purpose of stock enhancement of various fish species (Drawbridge 2002). Production and release of fertilised eggs and larvae became increasingly efficient as technology developed. However, there was no evidence to suggest that stock enhancement had an effect on the catches of the respective fish species. This was primarily due to a lack of emphasis on understanding the impact of stocking programmes on fisheries catches (Liao *et al.* 2003). Emphasis was focused, rather, on the quantity of fish released, under the assumption that this would reflect the effectiveness of the stocking programme. (Liao *et al.* 2003). In the 1940's stock enhancement efforts were terminated, as fisheries scientists began rejecting marine stock enhancement as an effective management tool (Liao *et al.* 2003). Emphasis shifted to aquaculture rather than stock enhancement until the 1980's, when stock enhancement again started gaining interest and critical questions regarding its effectiveness emerged as testable hypotheses (Liao *et al.* 2003). This period (between the 1940's and the 1980's) was termed the "denial phase" (Liao *et al.* 2003). Evaluation of the success of stock enhancement programmes became an important aspect, and methods such as tagging of cultured fish for release, as well as others discussed in Chapters 2 and 3 were developed. Several countries including Japan (Kitada 1999), Denmark (Rasmussen and Geertz-Hansen 1999), Sri Lanka (Davenport *et al*. 1999) and the USA (Lee and Ostrowski 2001, Serafy *et al*. 1999) are currently involved in successful stock enhancement programmes (Liao *et al.* 2003) and research into its use is being carried out in other countries such as Australia

(Taylor *et al.* 2005). Pilot scale stocking programmes for *A. japonicus* are also being conducted in New South Wales, Australia with fish being released into at least four different water bodies (Fisheries and Marine Environmental Research Facility 2003).

Due to the lack of evidence regarding the effectiveness of stock enhancement, the South African government has, until recently been reluctant to consider using it. However, with the growing global interest in stock enhancement, the South African government, too, is showing more interest in the potential that it offers. This has led to the development of a draft policy for marine ranching by Marine and Coastal Management as well as investigation into the stock enhancement of abalone, *Haliotis midae* (Godfrey 2003).

1.3.1. Objectives of stock enhancement

A stock enhancement programme can be categorised into one of three types; mitigation, augmentation or community change, depending on the fish species, the habitat and the objective (Cowx 1999, Mustafa 2003).

Augmentation stocking is undertaken to supplement the abundance of a species where production is perceived to be lower than the carrying capacity of the water body (Cowx 1999, Liao *et al*. 2003, Mustafa 2003), usually due to overfishing. The extent to which stocking can occur must remain within the limits of the habitat, without disturbing the ecological balance of the ecosystem (Mustafa 2003). The introduced stock should have a similar genetic make up to the wild stock in order to reduce the possibility of genetic contamination (Mustafa 2003).

Mitigation stocking is undertaken to compensate for a decrease in a fishery yield due to perturbations causing habitat destruction (Cowx 1999, Liao *et al*. 2003, Mustafa 2003). In this case the fishery is limited by the number recruits and stocking will need to be continuous as it is unlikely that a self sustaining population will be achieved, unless the perturbations are removed (Cowx 1999).

Community change is stocking an exotic species that is not originally from the local marine environment (Mustafa, 2003). This is a contentious issue as severe damage can be caused to the ecosystem (Mustafa, 2003). This will not be the case for *A. japonicus* so is not an issue in this study.

In the case of stocking enhancement of *A. japonicus* in South African waters, the proposed strategy would be augmentation stocking, to compensate for low spawner biomass until the spawner stock recovers. It would be used as a complimentary management tool in conjunction with the existing traditional management as shown in Figure 1.3.

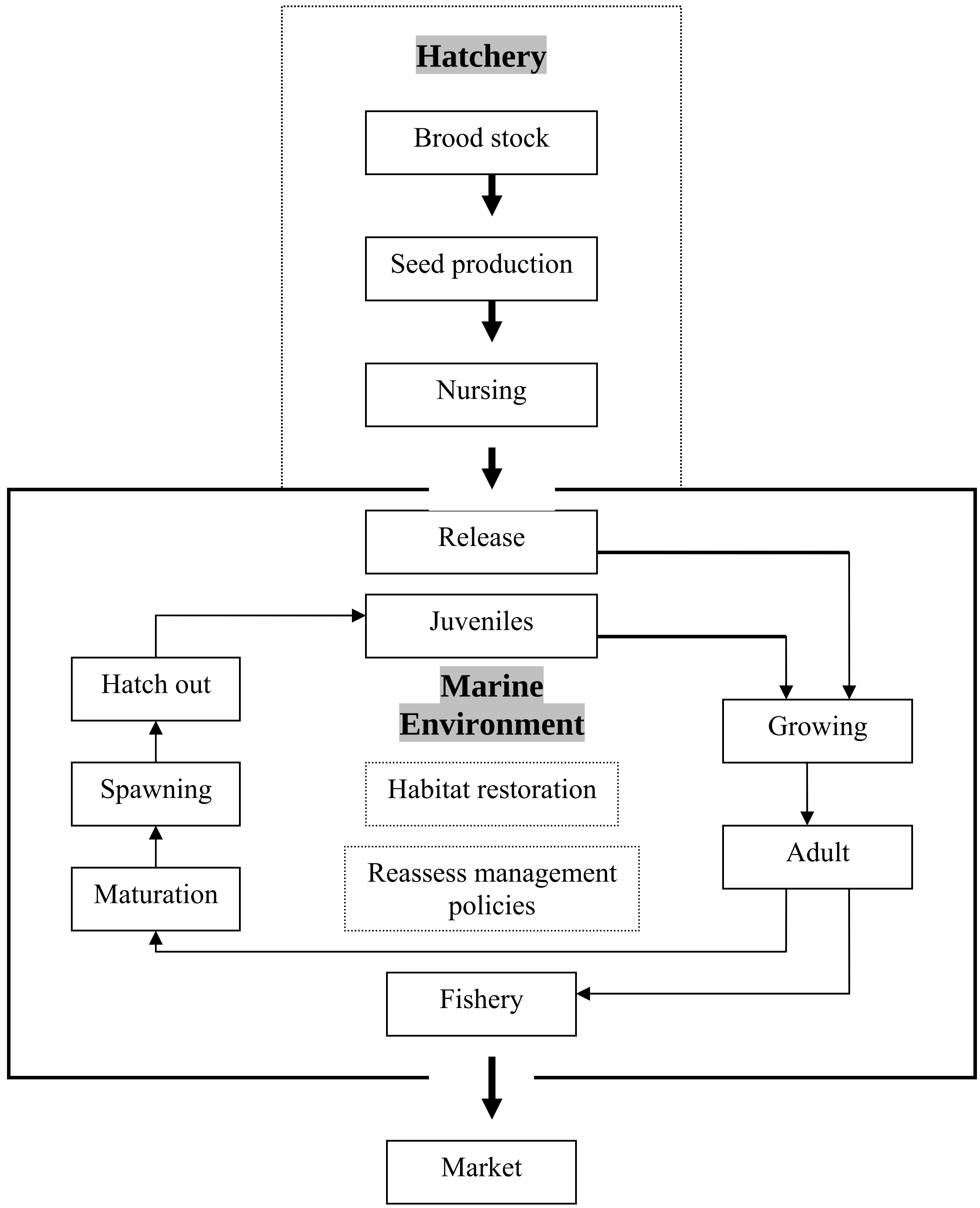

Figure 1.2: Recruit type stock enhancement (Mustafa 2003).

1.4. Stock enhancement as a management tool

Stock enhancement is often seen as a "quick fix" solution to dwindling fish populations (Molony *et al*. 2003). However, it is not as simple as it may seem, as there are several ecological and genetic implications to be considered (Chapters 2 and 3), and managers are obliged to apply the "precautionary principle" when making decisions regarding stock enhancement strategies. Managers must be sure that stock enhancement is an appropriate solution to rehabilitating a reduced fish stock. This can be done by asking a series of questions which require information about the stock (Figure 1.3). Bell (2004) suggests using stock enhancement when other management tools will take an unreasonably long period of time, or cannot facilitate the recovery of the species. One of the aims of the present project was to synthesise existing information relevant to the stock enhancement of *A. japonicus* and identify gaps in knowledge requiring further research.

Issues that must be investigated before a stock enhancement programme is implemented include legislation, risk assessment, management and release strategies. Traditional management tools such as bag limits, quotas, size limits, closed seasons and closed areas are relatively inexpensive and straightforward when compared to stock enhancement (Molony *et al*. 2003). These regulations are however difficult to control and with regard to most recreational line fish species in South Africa, have proven ineffective, resulting in the steady decline of many of these fish stocks (Brouwer *et al.* 1997).

There is often pressure on fisheries managers to use stock enhancement as a management tool as traditional management methods are usually unpopular among the users. With stock enhancement it is perceived that fish stocks will increase and at the same time fishing quality will improve (Molony *et al*. 2003). Managers should not be pressured into the use of stock enhancement for the incorrect reasons, and special procedures should be taken in the selection of the tool.

A stock enhancement programme that is not used in conjunction with traditional management tools runs the risk of creating a "put and take fishery", in which case stocking would need to be conducted on a permanent basis. However, the aim of any proposed stock enhancement of *A. japonicus* would be to augment the stock and not create a “put and take fishery”. It is for this reason that stock enhancement of *A. japonicus* should be seen as a complimentary management tool in an integrated approach to improve fish stocks (Molony *et al*. 2003) (Figure 1.3). The idea is that stock enhancement will help to restore the population quickly, but the cause of the problem should also be eliminated to prevent it from happening again once stocking has ceased (Howell 1998, Bell 2004).

Emphasis needs to go into the management of the enhanced stock using the already used traditional management tools. Because these measures are adequate to maintain stocks but not facilitate recovery, they should be enforced strictly. The costs associated with enforcement of regulations need to be included into the stock enhancement costs as it is essential to combine the two.

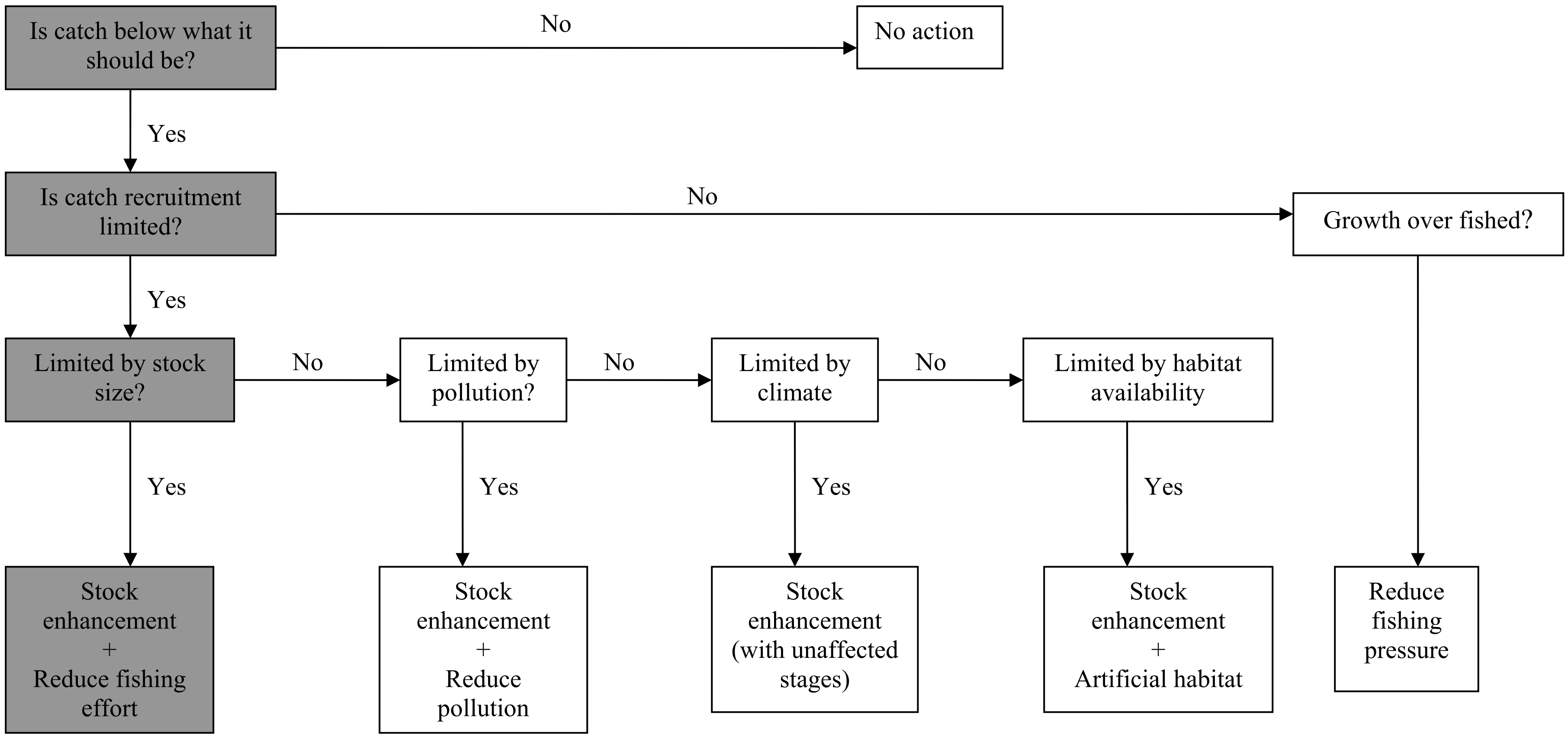

Figure 1.3: Steps taken when choosing an integrated approach to management of a stocking programme (Howell 1998).

1.5. Legislation and Policy

A sound legislative framework is essential to reduce the occurrence of the possibility of damaging enhancement practices as well as some of the common issues that arise from the stocking of fish, whether it is environmental, economic, or social issues (Howarth and Leria 1999).

In South Africa, stock enhancement falls under the jurisdiction of the Department of Environmental Affairs and Tourism. All aquaculture and restocking plans must therefore conform to the stipulated restrictions in terms of the Marine Living Resources Act (Act No. 18 of 1998) and the National Environmental Management: Biodiversity Act (Act No.10 of 2004). However, because research into the restocking of marine fish species in South Africa is in its infancy, there was until recently no policy on stock enhancement. A draft guideline for marine ranching was released for comment in 2006 by the Department of Environmental Affairs and Tourism. This study addresses several of the issues highlighted in these guidelines, in the relevant chapters, as indicated in the following list. The draft guideline states that proposals for stock enhancement will be analysed by a panel of experts in order to assess the risks involved, and should cover the following areas of concern:

1. Description of the proposed activity (Chapter 1)
    - Duration of the pilot phase
    - Details of the target species and associated biological parameters, including:
        - Growth
        - Reproduction
        - Survival rates
        - Resource status
    - Aquaculture facility for stocking must be approved
    - Description of proposed release site and release equipment and methods. Eg. timing, size/age at release

- Details of harvesting of released fish

2. Objectives and performance targets (Chapters 2, 3 & 4)
    - Monitoring methods
    - All releases animals must be marked

3. Economic feasibility (Chapters 5 & 6)
    - Cost benefit analysis
    - Details of facility, infrastructure and employment

4. Resource sharing (Chapter 5)
    - Addresses issues pertaining to distribution of benefits and how other users in the area will be impacted
    - Issues such as ownership of stocks and access rights must be addressed
    - Proposals where a wide group is benefited will be favoured

5. Environmental issues (Chapters 2 & 3)
    - Carrying capacity
    - Ecological
    - Genetic
    - Disease management

6. Monitoring (Chapters 2, 3 & 4)
    - Robust monitoring system must be implemented
    - Baseline survey should be conducted to assess status prior to stocking
    - Caution levels must be implemented
    - User groups, beneficiaries and liabilities need to be identified in the event of an unforeseen catastrophe

7. Compliance
   - A compliance risk assessment and plan should be provided to assess the risk of illegal harvesting of released stock and provide an approach to prevent that from occurring

8. Co-management
   - A co-management approach to management is suggested
     - This may be made easier by the fact that fish are being stocked rather than further restrictions being implemented

Pilot study

Once approval is given, a mandatory pilot study needs to be conducted to monitor risk assessment assumptions, as well as social and economic responses. This will also allow for scientific assessment of survival rates, causes of mortality, genetic impacts as well as some other environmental impacts. The pilot study is required to be long enough for the assessment of these effects, allowing enough time for the released fish to grow to harvest size, but should be small enough to minimise any negative effects that may occur (DEAT 2006)

Full scale stock enhancement

Once the proposal and pilot study have been deemed successful, and there is consensus that full scale stock enhancement can go ahead, the applicant may apply for a long term right, which may not exceed 15 years; the maximum length of a right allowed under the Marine Living Resources Act (DEAT 2006).

1.6. Risk assessment

Stock enhancement can be shown to be extremely effective when managed properly; however, as mentioned it has been shown in some cases to have adverse effects on the environment. An understanding of the impacts of stock enhancement is therefore necessary, and can be acquired through the use of systematic application of scientific method (Leber 1999), through which such effects may be controlled. In cases where acceptable levels of control cannot be achieved, stock enhancement should be rejected as a management option.

According to the draft stock enhancement policy released by Marine and Coastal Management, the environmental risks need to be assessed and monitoring needs to take place. There is therefore a need for an environmental risk assessment.

A clear understanding of these risks is necessary, and measures should be taken as far as is practicably possible to prevent or minimize them. To do this one needs to have an understanding of the water body, the biology, behaviour and ecology of the fish species involved, and the ecosystem as a whole, before one can understand the potential hazards that are involved in stock enhancement. This should be done prior to the implementation of the enhancement programme to decrease the likelihood of an ecological disaster.

In order to develop a responsible management plan for stock enhancement, these risks need to be identified. Further research should be conducted into the potential risks, and protocols developed that may decrease the effects and likelihood of the occurrence of ecological and genetic problems. However, the draft stock enhancement policy only provides a basic framework which must be built upon. Chapters 2 and 3 investigate and identify the actual content that would be required to plan and execute an environmentally safe stocking programme for *A. japonicus*, based on the requirements stipulated by the policy.

The risk estimation matrix (Figure 1.4) can be used to evaluate the risks of genetic and ecological problems associated with stock enhancement. The particular risks can each be assigned a level of impact and a level of likelihood within the matrix, from which risk estimation can be given. Measures can then be taken to either reduce the likelihood of the occurrence or the level of impact that the occurrence might have, thereby reducing the risk. An appropriate threshold level of risk, which should not be exceeded, must be agreed upon. In Figure 1.4, "very low risk" has been chosen as an appropriate level and anything above that (red) requires some measure to attempt to reduce it to below the acceptable level.

Risk estimation matrix

Likelihood of occurrence of risk	Negligible impact	Very low impact	Low impact	Moderate impact	High impact	Extreme impact
High likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate likelihood	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low likelihood	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low likelihood	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible likelihood	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequence of risk

Figure 1.4: Risk estimation matrix (Bartley *et al*. 2006).

Prior to any stocking of fish, a project application needs to be developed that incorporates the goals of the programme (discussed under the legislation heading), risk identification and likelihood estimate, identification of the possible consequences, and a management strategy that will minimise these risks (Figure 1.5). In order to achieve this, scientists require the information from the various studies discussed in the genetics and ecology sections of this chapter. Once gathered, this information should undergo a screening process as shown in Figure 1.6.

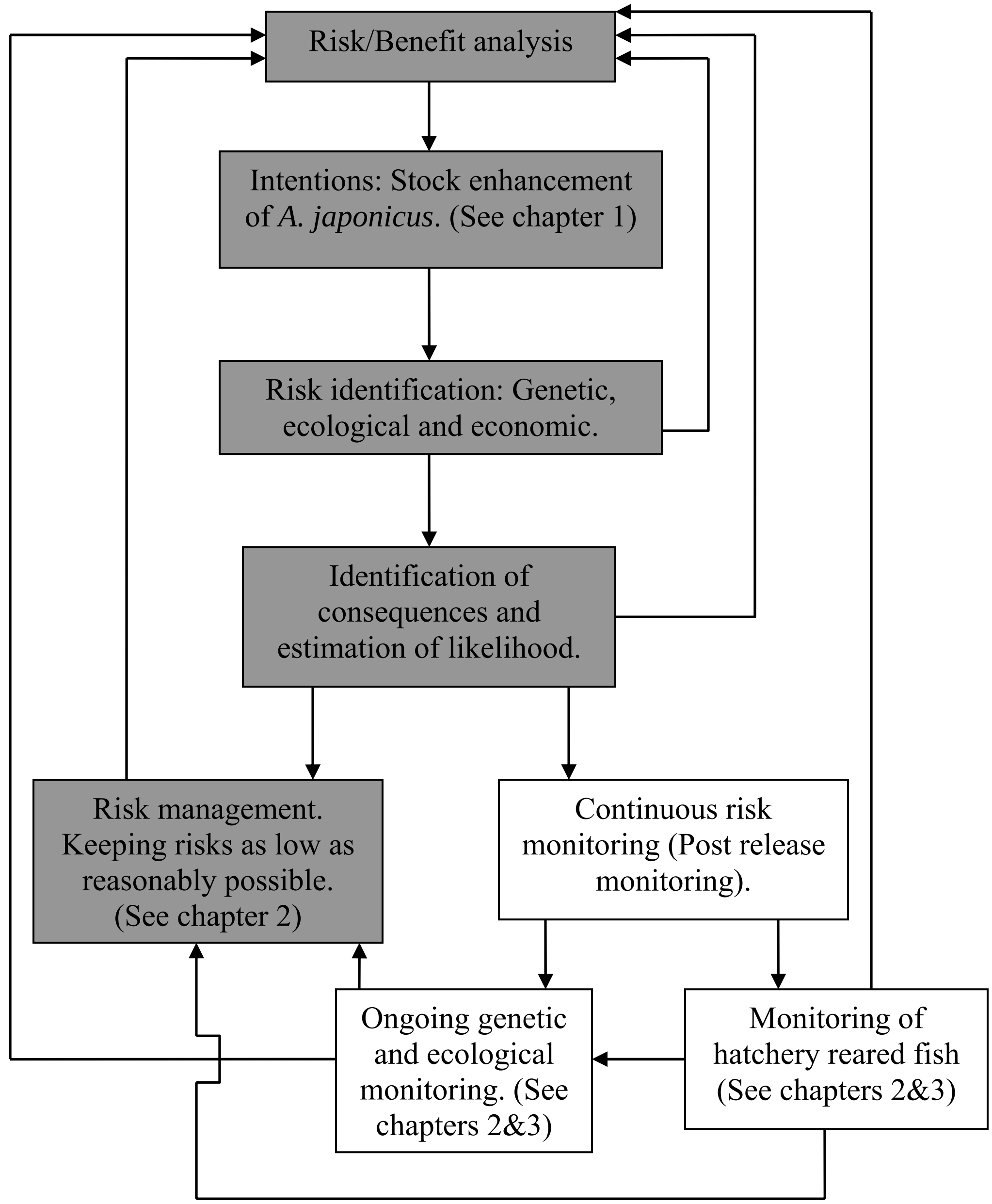

Figure 1.5: Risk assessment and management framework (Modified from DEAT 2002).

Screening is defined by Sadler (1996) as a process to determine whether or not a proposal requires further environmental assessment, and if so, what level of detail the assessment should include. The steps that need to be followed are illustrated in Figure 1.5.

The next step is to identify the risks involved in the project and the areas requiring further studies (Figure 1.5) in order to make informed management decisions based on sound understandings on the mechanisms behind the risks. With this information, management decisions can be made that keep the consequences of these risks at an acceptable level. These steps cover the pre-release prevention of predicted risks; however, management decisions also need to be made regarding the post release monitoring of fish. The management protocol would need to allow for rapid decisions and necessary changes to be made, which are relevant to the scenario presented. For example, if post release monitoring data shows that stocking has not increased the number of fish, then stocking should be ceased immediately. Decisions need to be made quickly in order to prevent adverse economic, genetic or ecological outcomes.

The genetics and ecology chapters (Chapters 2 and 3) review the risks associated with stock enhancement, and identify areas of research that can be used to develop management protocols that may mitigate the risks of stocking, which is the first step towards pre-application screening. A mandatory screening (Figure 1.6) can be undertaken once the required data has been analysed, and a thorough application containing all information, as described in this chapter. This may require further studies if there are still unanswered questions about the potential impacts of stocking.

After a management plan has been created, and screening processes are complete, and authorities grant permission to go ahead with the programme, continuous risk monitoring is required. The ecological and genetic risk monitoring is discussed later in this chapter.

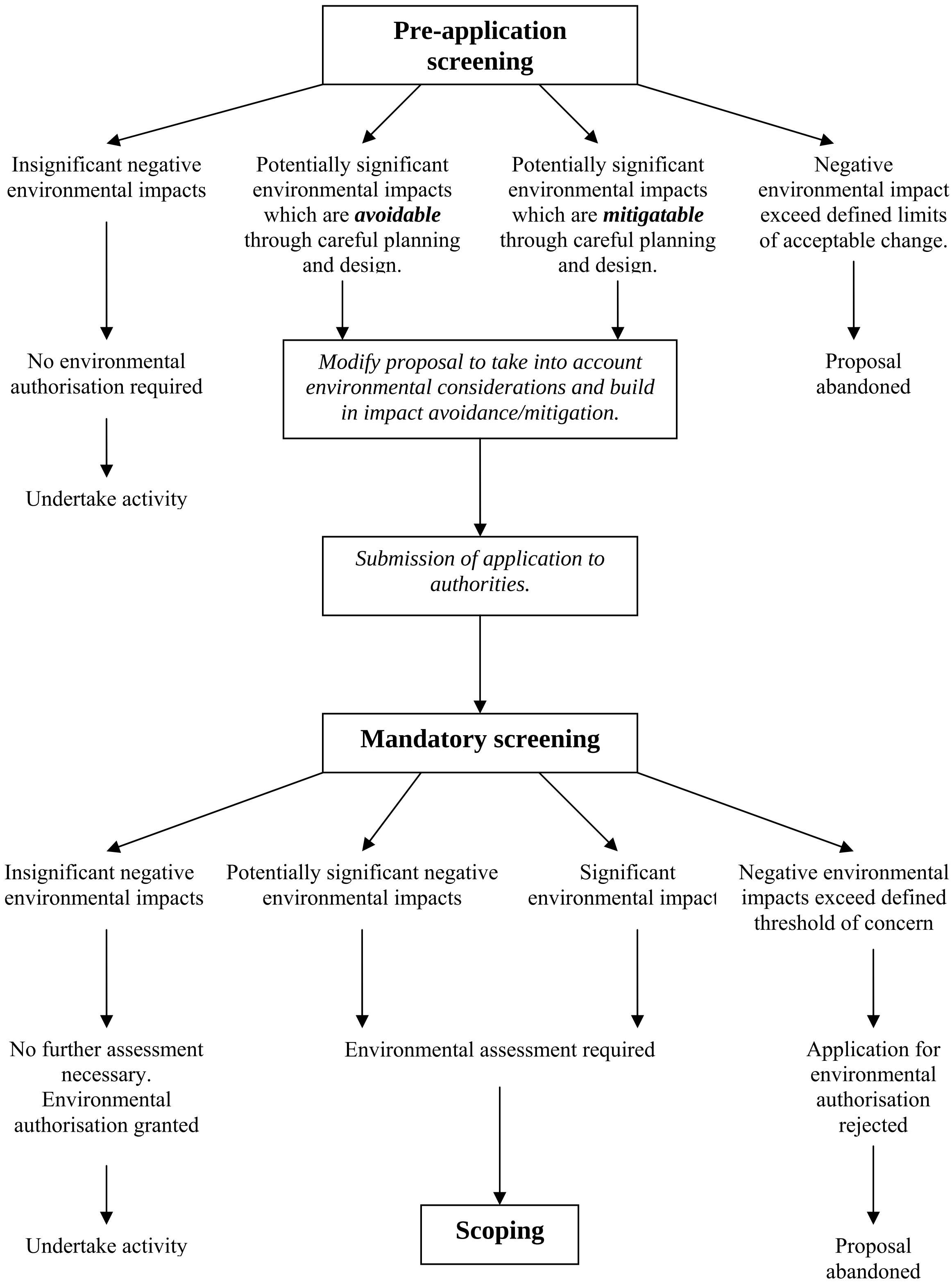

Figure 1.6: Overview of generic screening process (DEAT 2002).

1.7. Management of stock enhancement

"Marine resource management, including stocking, is often underpinned by largely untested ecological assumptions or hypotheses" (Liao *et al*. 2003, 157). As fisheries management shifts its focus towards long term sustainability, as opposed to open access to the resources, science is required to deal with the complex issues that arise. Attention has now turned towards the environmental concerns involved in stock enhancement, particularly genetic and ecological issues. Fish culture technology has improved to a point where the potential for carrying capacity limits, and adverse ecological and genetic effects, have become the primary biological limitations on stock enhancement (Hilborn 2004) (Chapters 2 & 3). Stock enhancement programmes are often conducted without consideration of broader fisheries management aspects, and often the aims of research have only been towards the low cost production of juveniles for release and increased survival of these fish (Bell *et al.* 2006). The development of a management plan is necessary to provide guidelines for the effective use of stock enhancement of *A. japonicus*. To develop this management plan, research is still needed to understand the interactions among stocked organisms and the wild population (Leber *et al*. 2004).

Caution is required when planning and implementing a stock enhancement programme as many uncertainties remain regarding the successful use of this management tool. Risks stem from inadequate decisions about hatchery and release protocols as well as the timing and location (Leber *et al*. 2004).

If stock enhancement is to be used as a management tool for *Argyrosomus japonicus* in South Africa, careful planning will need to be carried out to design a responsible programme, where results show maximum effectiveness with minimal negative impact on the environment. The development of a responsible management plan requires information on rearing techniques, release strategies, monitoring, disease defence, and evaluation of hatchery effects on wild stocks (Leber *et al*. 2004).

Blankenship and Leber (1995) provide guidelines for a responsible approach to stock enhancement. The guidelines include ten components, each forming an integral part of the development, evaluation and management of a marine stock enhancement programme. Several of the points in these guidelines appear in the draft policy for stock enhancement in South Africa (DEAT 2006). The full set of guidelines proposed by Blankenship and Leber (1995), and the extent to which they are addressed in this thesis, are as follows:

- Establish methods for prioritizing and selecting species to be enhanced. (Chapter 1)
- Estimation of the shape and size of the fish stock in question.
    - This provides useful information regarding the need for stock enhancement
    - Can be used to predict the effectiveness of stock enhancement
    - Can be used to calculate the number of fish required to be released in the stocking programme
- Create a management plan with long and short term goals, suitable harvest regimes and genetic conservation objectives (Chapter 3)
    - Identify harvest opportunities, stock rebuilding goals and genetic objectives
    - Consider goals and objectives in the context of the management for candidate species
    - Evaluate management strategies to use in conjunction with stock enhancement
- Incorporate an understanding of life history and ecological attributes into enhancement strategies and tactics. (Chapter 1)
- Create a genetic resource management plan to minimize inbreeding/ outbreeding depression and to conserve genetic resources (Chapter 3)
    - Identify the genetic risks and consequences of enhancement
    - Define an enhancement strategy
    - Outline research needs and objectives
    - Develop a feedback mechanism

  - Implement genetic controls in the hatchery and a monitoring and evaluation programme for wild stock
- Create a disease and health management plan. (Chapter 2)
  - Adopt responsible hatchery practices
  - Certify fish are free from viral, parasitic and bacterial infections
- Define and use an empirical process for determining optimal release strategies. (Chapter 2)
  - Evaluate stock enhancement in an ecological context
  - Evaluate behavioural and physiological deficiencies that may be present in stocked fish
- Define and implement means to identify hatchery produced fish. (Chapter 4)
- Define quantitative measures of success, and assess the enhancement project in terms of stated objectives in the management plan.
- Define and evaluate socio-economic objectives. (Chapter 5)
- Use adaptive management principals to evaluate and improve management strategies and tactics.

Each of these components is considered to be essential in controlling and optimising the effectiveness of a stock enhancement programme (Blankenship and Leber 1995) with minimal negative impact on the environment. These components are considered in the relevant chapters of this study as indicated in the above list.

1.8. Aims and Objectives

This project aims to assess the feasibility of using stock enhancement for the management of *A. japonicus* in South Africa, based on available scientific information and accepted principles for responsible stock enhancement. The development of a management framework and its application for the stocking of *A. japonicus* in South Africa requires that a substantial amount of research be carried out. Chapters 2 and 3 identify the risks involved in stock enhancement of *A. japonicus* based on its known biology, and make suggestions on how to minimise them, and areas requiring further research in order to minimise risks are identified. An essential requirement for research is the identification of fish after release. Chapter 4, therefore, identifies a suitable tagging method that can be used in juvenile *A. japonicus*.

The economic feasibility is a major factor in determining whether a management tool is appropriate. Chapter 5 uses a willingness-to-pay survey among recreational anglers to estimate the potential income that could be generated from stock enhancement of *A. japonicus*, while chapter 6 provides a costing model for stock enhancement. Using the information from these two chapters, the economic feasibility of stock enhancement of *A. japonicus* is modelled.

CHAPTER 2

ECOLOGICAL ISSUES RELATED TO STOCK ENHANCEMENT OF *ARGYROSOMUS JAPONICUS* IN SOUTH AFRICA

"Few, if any, human interventions in the environment fail to have impact. In some cases interventions are potentially so damaging that they must be eliminated. On the other hand, the majority of human interventions are purposeful and designed to be of benefit to humans, so it is necessary that they proceed responsibly… (Nash *et al*. 2005)."

2.1. Introduction

The nature of stock enhancement is such that an ecosystem is altered due to the introduction of hatchery reared fish. As a result of this there is a possibility of negative ecological effects on the environment. However, the effects of stocking can be controlled to a certain extent through responsible management techniques, and positive effects can be maximised while negative effects minimised.

When it reaches the stage that stock enhancement is required, it is usually because the ecosystem has been disrupted in some way, usually from habitat destruction or overfishing. Stock enhancement can then be a useful tool to rectify the problem. However, ecosystems are very delicately balanced and can be easily disrupted by human disturbances. There are therefore unavoidable dangers that that can arise from the stocking of hatchery reared fish into an ecosystem (Waples and Drake 2004). Through careful management procedures, these risks can be minimised to acceptable levels (Figure 2.1). Figure 2.1 provides a guide to the necessary steps for developing an ecologically safe stock enhancement programme and is the framework upon which this chapter is based.

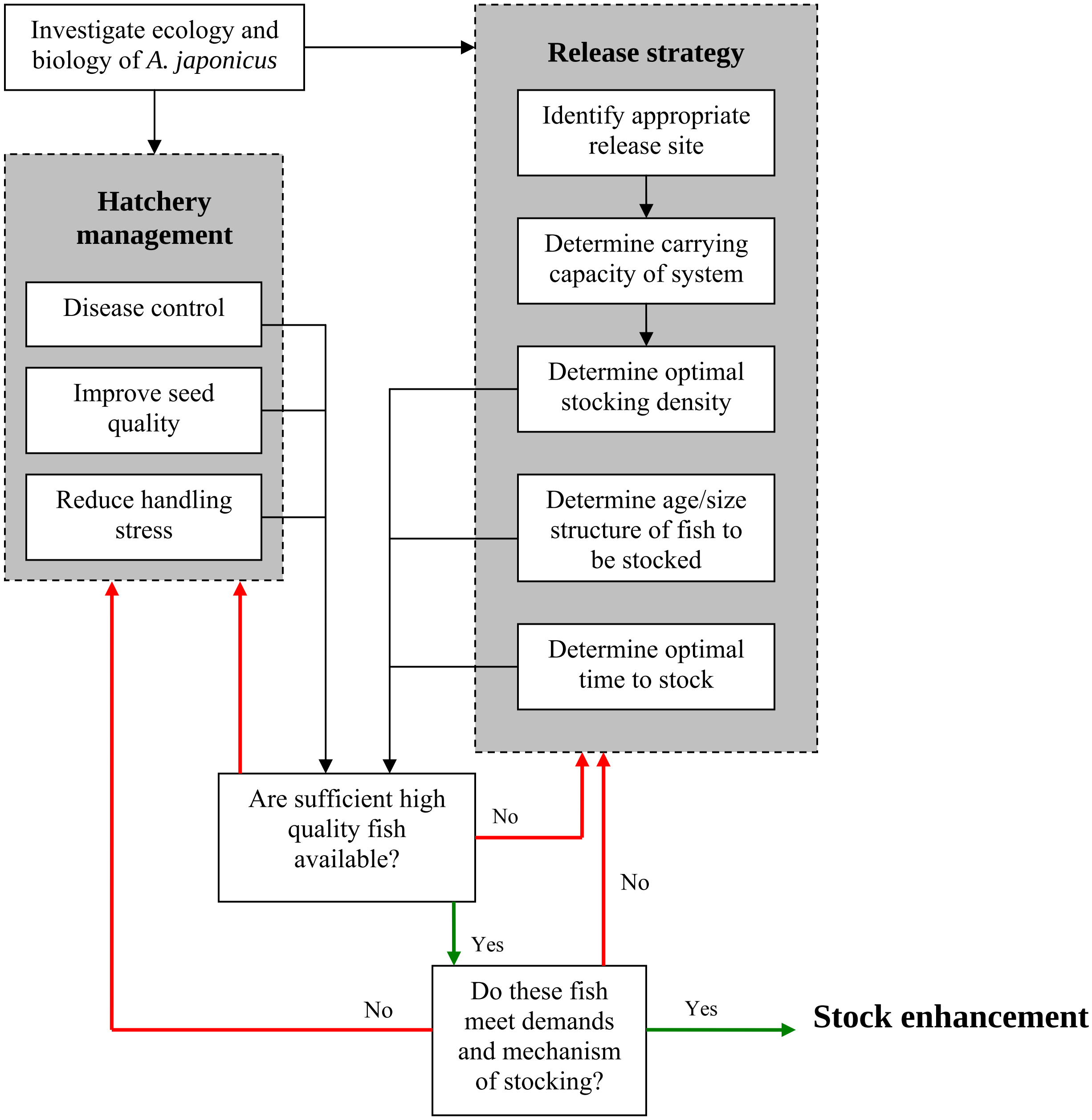

Figure 2.1: Flow chart illustrating necessary ecological considerations when planning a stock enhancement programme.

Due to the potential for ecological problems to occur as a result of stock enhancement, research is required to develop a stocking programme that is ecologically acceptable. To do this the nature of a stock enhancement programme should be largely dictated by the biology of the species being stocked. For example, broodstock selection should be based on information on the ecology of wild mature *A. japonicus*, while rearing and release strategies should be based on juvenile ecology.

Given that *A. japonicus* has a relatively complex life history strategy and are predators in an ecosystem, the impact caused by the alteration of their numbers by stocking could be severe. It is for this reason that release strategies should be considered prior to stocking. The considerations that should be taken into account are the number and size of the fish to be released and where they should be released, as well as the genetic considerations which are discussed in chapter 3.

2.2. Release strategies

2.2.1. Number of fish to be released

The number of fish that can be released in a stock enhancement programme is a trade-off between stocking too few fish, which is ineffective in enhancing the stock, and too many fish which may cause disruptions in the ecosystem. Both ends of the scale need to be investigated and quantified so that stocking can be done in an effective, non destructive manner.

Carrying capacity is defined by Liu (2004; 1) as "the maximum number of individuals that a given environment can support indefinitely, without detrimental effects to environmental state." If the carrying capacity is exceeded, populations of other organisms within the ecosystem can become locally extinct and the environment could be permanently altered or destroyed. It is difficult to calculate the carrying capacity of an ecosystem with respect to a particular species as it is not a fixed value. Carrying capacity changes from season to season and from year to year according to abiotic (e.g. climate) and biotic (available food, predators, competition, etc.) factors (Liu 2004).

Lui (2004) has developed a conceptual model for estimating the carrying capacity of a site for stock enhancement and the adjacent area (Figure 2.2). The first step is selection of the ecosystem habitat that the released fish will utilise. In the case of juvenile *A. japonicus*, this is the upper, middle and lower regions of estuaries and the adjacent inshore area (Griffiths 1997b) which are their natural habitat. The elements that affect the carrying capacity are the abiotic factors such as the habitat type, and the biotic factors which are the direct or indirect interactions between *A. japonicus* (both wild and hatchery reared) and other organisms. These factors can then be used to create a mathematical model that will attempt to simulate the interactions between the factors, from which the carrying capacity of the ecosystem can be estimated. These types of models have been used by Pulatsu (2003) and Luo *et al.* (2001) to estimate carrying capacity, however, models will vary with species and habitat so will need to be designed specifically for *A. japonicus* in the estuarine and marine environments.

The first step towards estimating the required numbers of juvenile *A. japonicus* for stock enhancement is to estimate the shape and size of the wild stock. By doing so managers will get an idea of the numbers of fish that will help to facilitate the recovery of *A. japonicus* stocks. Knowing the number of fish that need to be stocked is important, not only to avoid ecological problems, but also in estimating the economic feasibility of such a stocking programme. The importance of estimating the shape and size of the stock is recognised, however, due to financial constraints was not within the scope of this study. It is, however, recognised as one of the first steps towards stock enhancement of *A. japonicus* in South Africa.

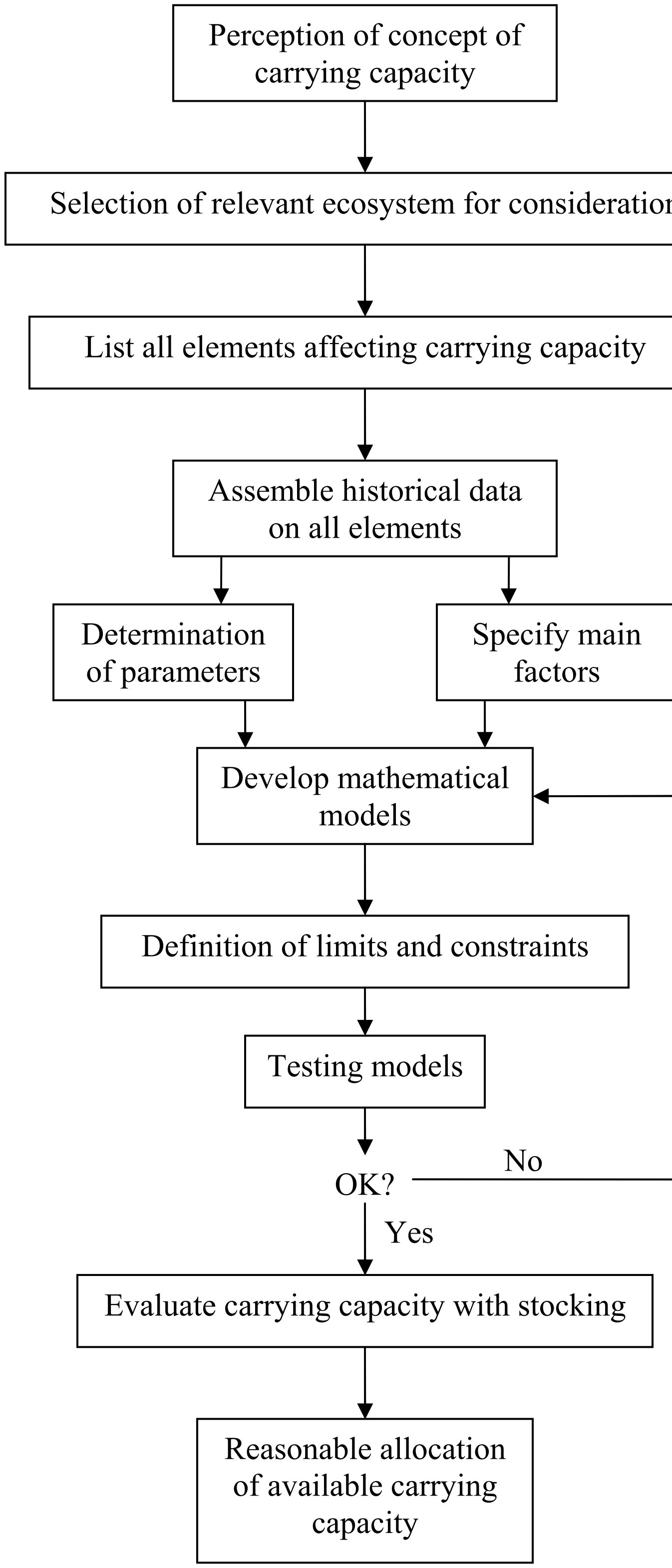

Figure 2.2: Flow chart for estimating carrying capacity with respect to stock enhancement (Modified from Lui 2004).

The fundamental concept behind stock enhancement is where the upper trophic levels (in the case of *A. japonicus*) have been over exploited and are augmented by stocking of hatchery reared individuals (Figures 2.3(a) and 2.3(b)) . Figures 2.3(a) and 2.3(b) show that there is a limit to the number of fish can that be stocked, which is a function of the productivity of the trophic level beneath it. Overstocking and hence exceeding the carrying capacity would deform the pyramid causing an unbalanced ecosystem which could have deleterious affects on many organisms from different trophic levels in the ecosystem (Figure 2.3(b)).

The need for stock enhancement of *A. japonicus* is due to over exploitation of the stock and subsequent decline in the population's reproductive potential. *A japonicus* stocks are heavily depleted with spawner biomass per recruit lower than 4.5% (Griffiths 1997a). Thus, it is unlikely that overall carrying capacity of the ecosystem would be exceeded in the early stages of a stock enhancement programme. However, due to the species' utilisation of estuaries as nursery areas during the first year of their lives, the risk of exceeding the carrying capacity in the estuaries used as stocking sites is real and should be monitored carefully. Over-stocking can also occur if the enhancement programme continues once the stocks have recovered (Waples and Drake 2004).

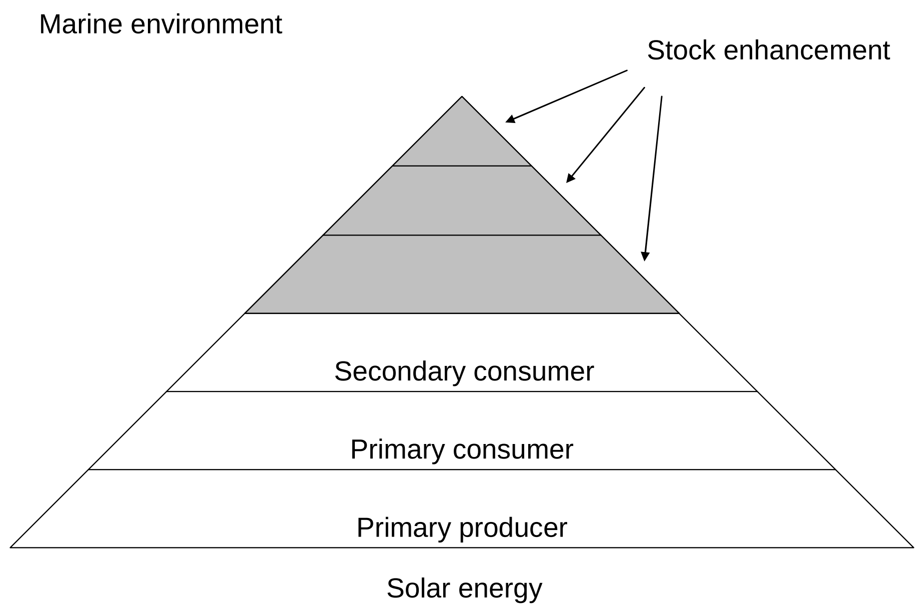

Figure 2.3(a): Ecological pyramid in the marine environment. Productivity at any level of the pyramid is limited by the level below it. Because *A. japonicus* belongs at a high level of the pyramid, the number of juveniles released cannot exceed the limits of the level below (Tsukamoto *et al.* 1999).

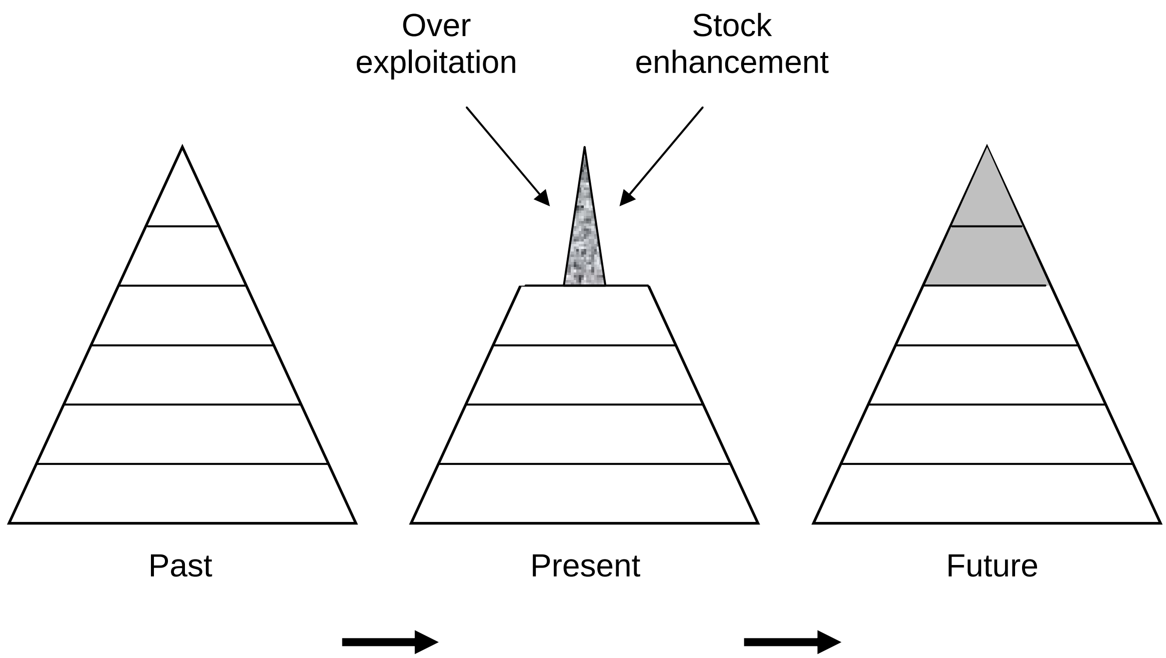

Figure 2.3(b): Pyramid deformed by over exploitation. Severe exploitation as in the case with *A. japonicus* yields unutilized capacity near the top of the pyramid which can be repaired with responsible stock enhancement (Tsukamoto *et al.* 1999).

2.2.2. Optimum size at release

Releasing fish at an optimum size is one of the most important factors in creating a stock enhancement programme that is effective, as this is closely associated with production costs and severely influences the survival of the released fish. The longer the fish are kept in the hatchery the more costly it is, but survival among released fish is increased causing a trade-off between cost and survival (Ottera *et al*. 1999, Mahnken *et al*. 2004). Mortality is reduced by releasing juveniles at a larger size when they are less susceptible to predation.

However, release size should not be based purely on a cost benefit between survival and production costs. Smaller fish that are released, and are subjected to natural selection can create a stronger overall population of released fish as the weaker individuals will not contribute to the wild population. Therefore, the release of smaller fish and hence higher natural mortality will minimise the genetic consequences of unnaturally high survival in the hatchery and genetic effects of "hatchery selection".

Estimations of natural size dependent mortality can be used for preliminary estimations of production costs and effectiveness of the programme, but to maximise efficiency of the programme, more accurate calculations should be done. Release trials with hatchery reared fish can be used to calculate the optimum size at release, but should be the last step towards optimising the effectiveness of the hatchery, once all other precautions that have been discussed have been taken.

Yamashita and Yamada (1999) performed a trial to determine optimum size for release by releasing groups of various size classes of juvenile Japanese Flounder (*Paralichthys olivaseus*). The fish were marked with an otolith dye (unspecified) shortly before release. Otoliths of recaptures were then examined under ultraviolet (UV) light, and by comparing the diameter of the fluorescent mark and the length of the fish, it could be determined at what size the fish had been released. Frequency of recapture of the different size classes was compared and optimum size was determined, where frequency of recaptures of each particular size class acts as a proxy for survival.

Such a study would be well suited to *A. japonicus* as they have been shown to be tolerant of and exhibit excellent tag retention with Oxytetracycline-HCL (OTC), a fluorescent dye for marking otoliths (Chapter 4 of this study, Taylor *et al*. 2005a). The fact that *A. japonicus* spend the first year of their life in the estuary and adjacent area makes for simpler monitoring and recapture of these fish.

Stocking should necessarily take place in estuaries rather than in the ocean as the minimum size class of *A. japonicus* suitable for release is within that size range that wild *A. japonicus* are in their estuarine phase. Recruitment into the estuary occurs when juveniles are between 20 mm and 30 mm (TL) (Griffiths 1996). Stocking with smaller fish is not technically feasible as transportation of hatchery reared *A. japonicus* at a size of less than 25 mm to 30 mm (TL) results in unacceptably high stress related mortality

(Guy Musson, Espandon Marine, pers. comm. January 2007). There is also no reason to believe that small (< 20 mm (TL)) hatchery reared fish that are released into the sea would recruit into estuaries.

2.2.3. Handling stress

Prior to and during stocking, fish are inevitably subjected to stress. Capture from the holding facility, transport and release of the fish into the wild all play a role in stressing the fish to some degree (Serafy *et al*. 1999). Studies have shown that these types of stressors can lead to osmoregulatory dysfunction, lowered disease resistance, reduced swimming performance and behavioural disorientation (Serafy *et al*. 1999). These stressors all have obvious implications for stock enhancement as they reduce the fitness of the released fish and hence decrease their chances of survival, and may too themselves be lethal. A study by Serafy *et al*. 1999 on red drum (*Sciaenops ocellatus*), to determine whether cumulative stressful activities on the same day resulted in higher handling mortality than the same stress over several days, showed that same day stresses resulted in lower mortality (Serafy *et al*. 1999). A similar study should be conducted with *A. japonicus* as it is important to minimise any factors that may lead to reduction of fitness among released fish.

2.3. Aspects of ecology relevant to stock enhancement

2.3.1. Competition/predation

Competition, both interspecific and intraspecific, between hatchery reared fish and wild fishes is of concern (Waples and Drake 2004). Released fish, in their unnaturally high densities, especially near the release sites, will compete for food and space with both conspecifics as well as other species. This should be monitored and managed by careful determination of a suitable number of release sites, and a suitable number of fish to be released at each sites. If not managed correctly, this can have a damaging effect on the ecosystem as opposed to enhancing the wild stocks. Considering the highly dynamic and productive estuarine environment, into which the fish would be stocked, it is unlikely that stocking juvenile *A. japonicus* would lead to major long-term complications with regard to competition. However, this does need to be investigated.

As *A. japonicus* is a predator, sudden influxes during stocking would lead to increased pressure on prey species, both near to the release areas and in the adjacent ocean habitat as the fish grow up. However, as the *A. japonicus* stock is so severely depleted (<4.5% spawner biomass per recruit), ecosystems are likely to be well below their carrying capacities and the addition of the predatory *A. japonicus* should not cause a major problem. The possibility of these competition-related problems associated with stocking, however, should not be overlooked and continuous monitoring should take place. Ichthyofaunal studies should be conducted at the release sites and adjacent areas prior to stocking and should be repeated regularly throughout the duration of the stocking programme. As *A. japonicus* utilise estuaries as nursery habitats during their early juvenile stages (Whitfield 1998), estuaries would need to be used as release sites. This makes post release monitoring far simpler as fish can be monitored for up to a year in the release estuary.

<u>2.3.2. Predation</u>

The general theory for predator-prey relationships is that an increase in prey leads to an increase in predators (Sægrov and Skilbrei 1999). Although *A. japonicus* is a predatory fish, the juveniles are prey for a number of piscivorous fish and bird species. The instantaneous increase in numbers of juvenile *A. japonicus*, due to an enhancement programme could lead to an increase in the numbers of predators, preying not only the hatchery reared individuals but also on wild juveniles as well as on other species (Sægrov and Skilbrei 1999). However, estuaries are rich habitats with many species of fish in high abundance of similar size to juvenile *A. japonicus*. Furthermore, *A. japonicus* are relatively cryptic in terms of behaviour and appearance, minimising the chances of attracting large numbers of predators.

Although it may not be a problem with juvenile *A. japonicus* as they recruit largely into more turbid estuaries to avoid predators, predation by piscivorous fish and birds can contribute a large proportion of mortality among newly released juvenile fish (Serafy *et al*. 1999). Predation by birds can easily be reduced by chasing the birds away, during, and for a short period after stocking, while the released fish are aggregated near the release site, or by releasing fish at times when birds are not feeding (e.g. after dark). In the case of piscivorous fish predation may be more difficult to monitor, but can include examining the gut contents of predatory fish in the vicinity of the release site as well as estimating the number of predators present (Serafy *et al*. 1999). Releasing fish into protected areas or areas where predators are less prevalent or at times of the day when predators are less prevalent or not feeding can minimise the effects that predators may have on the productivity of the stocking programme (Hossain *et al*. 2002). This is particularly useful for the stocking of *A. japonicus* as the release site is most likely to be in an estuarine environment, where predators are less prevalent.

The main reasons for high mortality in the newly released fish are that they may not be accustomed to the presence of predators, and thus may not exhibit avoidance behaviour; compounded by the stresses incurred during their release, affecting their ability to escape. Conditioning can be used in the hatchery to prepare fish for the wild conditions that they will be exposed to after release (Mahnken *et al.* 2004, Masuda 2004). Conditioning can eliminate or reduce abnormalities in behaviour, physiological, developmental, ecological, and environmental and feeding deficits. For example, it has been shown by Kuwada *et al*. (2000) and Maynard *et al.* (2001) that fish can be trained to stay in sheltered areas therefore avoiding predators. It has also been shown by Olla and Davis (1989), Kellison *et al*. (2000) and Hossain *et al.* (2002) that fish that had encountered predation pressure exhibited better survival than naïve fish that had not been exposed to such pressures. Nodtvedt *et al.* (1999) also showed that cod that had been exposed to predator recognition training kept a greater distance from predators than untrained individuals. This “learned” behaviour of predator avoidance is a valuable tool to consider for use in the hatchery and could be used for other behaviours such as prey capture ability. Studies would, however, need to be done with *A. japonicus* to identify behaviours that may require training and also determining the effectiveness of the training on the species.

It is important that predation be monitored as it provides valuable information regarding the initial survival of the released fish and can play a major role in determining the appropriateness of the release site and release time.

2.3.3. Disease

Infectious diseases have been known to cause mass mortalities among larval and juvenile fish since the beginning of mass seed production. The main causes of this are degradation of environment and proliferation of pathogens under hatchery conditions (Liao *et al*. 2003). The risks brought about by disease in the hatchery can lead to numerous problems: 1) low survival in the hatchery, reducing efficiency of the stock enhancement programme; 2) unnatural selection pressures, producing an unrepresentative cohort of fish for release; 3) release of fish that are carrying pathogens which can be spread to wild fish. Disease control is therefore a top priority for a stocking programme as it can limit the effectiveness of the entire programme.

The spread of disease within a hatchery can occur in several ways, including a contaminated hatchery, horizontal transmission from infected fish, vertical transmission from infected parents to offspring, food, blood transmission by blood feeding vectors, and from poor nutrition (affecting the immune system) (Bartley *et al*. 2006). All of these aspects should be considered by hatchery managers and necessary precautions taken to minimise the risk of disease transmission and outbreaks within the hatchery.

The main health problems encountered by *A. japonicus* hatcheries include ciliated protozoan and monogenean trematodes (Anon. 2001). Controls for both protozoa and trematodes exist, and can be achieved through the use of prophylactics or treated as required (Anon 2001).

Most pathogens originate from wild stock, but only become pathogenic under intensive aquaculture conditions. Therefore reared fish that may be carrying a low parasite load and show no signs of morbidity on release, are unlikely to cause problems to wild fish as the pathogen will be less harmful in the wild due as a result of the natural stocking density.

In view of the importance of disease management, Bartley *et al*. (2006) have highlighted the steps that should be implemented to manage:

1. Source of animal to be released
2. Population to be managed
3. Hazard identification
4. Risk management
5. Quarantine
6. Diagnostic and treatment procedures
7. Mitigation measures
8. Monitoring
9. Reporting disease status of hatchery and wild populations
10. Estimation of aquatic animal health standards

Mitigating the potential risks of disease introduction, spread and establishment must take place in both the hatchery and the natural environment; mitigation in the hatchery being simpler than in the natural environment.

Control measures have made it possible to produce disease free juveniles in hatcheries. One may automatically think that this would lead to a higher survival rate; however, as these juveniles have not been exposed to viruses, they may show increased susceptibility to viral pathogens after release, causing lower survival rates (Mushiake and Muroga 2004). Mushiake and Muroga (2004) propose several control measures that can be used in order to reduce the risk of infections in the hatchery as well as after release:

Methods of Control

1. Avoid exposure to the pathogen
2. Environmental manipulation
3. Vaccination
4. Activation of innate immunity
5. Chemotherapy

6. Breeding for disease resistance
7. Health management

Although some of these control methods are advanced and do not all appear practical for an early stage stocking programme at this time, others are more realistic options, and certainly with further investigation and the improvement of technology, may become realistic in the future. But for now managers have available to them, options which are well within the constraints of funding and technology.

Quarantine is a primary form of disease control that should be practiced in a hatchery of any type. Broodstock fish should be kept isolated in terms of tanks and system water for a suitable period of time, and tested for known pathogens. This reduces the risk of introducing disease into the hatchery via new fish that are brought in (Bartley *et al*. 2006). Good husbandry and hatchery practices such as nutrition, rearing densities and broodstock management may also reduce the risk of disease outbreaks. Fish batches which have exhibited high mortalities should not be considered for release while those from apparently healthy batches should be tested for known pathogens prior to release. This type of visual observation can be valuable in the prevention of the spread of disease.

Once a pathogen or disease becomes established in an environment, it is difficult to eradicate it, and management should, therefore, aim at avoidance of the risk. "A pathogen in a fish or in nature is not indicative of a disease condition. Disease is a complex interaction between the host, the pathogen and the environment." (Bartley *et al*. 2006). Avoidance can therefore, take place at any of these levels. Degraded habitats have often been the sites of disease outbreaks. These sensitive areas should be avoided as stocking sites as they are predisposed to disease transmission from the culture environment to the natural environment. Although caution should be observed, problems are unlikely to occur, as only broodstock from the local ecosystem would be used. However, if problems do arise, there are alternate options.

The use of vaccines is becoming increasingly popular in aquaculture and has been used in stock enhancement (Buchmann *et al*. 2001). Vaccines reduce the impact of the disease and hence reduce the need for antibiotics. No residues are left in the fish or the environment and resistance by the pathogen is not induced (Bartley *et al.* 2006), as can be the case with antibiotics. However, because the effect of the disease is reduced, stocks of disease carrying fish can be produced, placing wild fish at risk (Bartley *et* al. 2006). This occurs in cases where fish that "should" have died from a certain disease, survive and are able to transmit the disease to other fish.

Selecting for disease resistant fish is common practice in commercial aquaculture, but may not be an efficient disease management tool in stock enhancement programmes, as genetic variability is therefore reduced. Furthermore, selected genotypes may be fitter in the hatchery than in the wild, or selected genotypes could be lost when breeding with wild fish occurs (Bartley *et al*. 2006). Selection pressure on hatchery reared fish (intended for stock enhancement) is discussed in the genetics section of this chapter. The management of diseases should incorporate principles from the genetic management so as not to cause genetic problems while eliminating diseases.

2.3.5. Seed quality

The fitness of individuals intended for release is a key factor for a successful stock enhancement programme, as there is a correlation between the quality of juveniles that are released and their chance of survival in the wild (Svasand 2004). The potential to improve seed quality and hence stocking efficiency is an area of research that should be investigated for candidate species for stock enhancement (Leber *et al*. 2004).

Certain morphometric characters in hatchery reared fish can differ from those in wild, due to differences in the environmental conditions that the fish are exposed to. Various abnormalities due to artificial rearing conditions have been shown in numerous species including: bone abnormalities in red sea bream (*Pagrus major*) (Matsuoka 1987), malformations of the lateral line in gilthead sea bream (*Sparus aurata*) (Carrillo *et al*.

2001), and inadequate body pigments or colour change (Blaxter 1975, Fairchild and Howell 2004); all of which can lead to reduced fitness among released fishes.

Ecological differences between the hatchery and the natural environment can also lead to behavioural problems in hatchery reared fish. As this can play a role in feeding as well as predator avoidance, survival among these fish can be compromised (Fairchild and Howell 2004, Svasand 2004). In the case of a stock enhancement hatchery, behaviour can be altered by two mechanisms; 1) experience, and 2) mortality and survival of different behavioural phenotypes (Huntingford 2004). Behavioural differences between cultured and wild fish stem from the differences between the two environments. The hatchery environment can be less challenging to developing fish in that high quality food is readily available, eliminating the need to track food, there is a lack of predators, fish are often treated for diseases, and the lack of space in the hatchery makes migratory behaviour impossible (Huntingford 2004). On the other hand the hatchery may be more challenging to juvenile fish as there is frequent disturbance by humans, high stocking densities which increase incidences of social encounters (often aggressive or cannibalistic), and food may be delivered in such a manner that promotes competition (Huntingford 2004). These can all affect behaviour in the juvenile fish that may continue after release into the wild and hence compromise the fitness of the fish.

Svasand (2004) suggests that the occurrence of morphometric and behavioural abnormalities in hatchery reared fish can be minimised through the use of natural (extensive) rearing systems. By maintaining the rearing conditions for hatchery reared fish as close as one can to those encountered by their wild counterparts, the effects responsible for the abnormalities may be reduced. A step toward this would be the use of structurally complex rearing tanks (Huntingford 2004). Studies involving the comparison between wild and cultured *A. japonicus*, both prior to and after release would provide useful information (Masuda 2004) that is necessary for the development of a hatchery management plan. Although there is no evidence to suggest that the abnormalities seen in the juvenile hatchery produced *A. japonicus* in Figure 2.4 are a result of the conditions in the hatchery, they do deserve investigation. The abnormalities seen in Figure 2.4 are likely to be a natural genetic phenomenon, unrelated to the hatchery environment, and such fish will be selected against in the wild, due to their reduced fitness. If abnormalities are found to be a natural genetic phenomenon and are restricted to a small number of fish then it is not a problem, however, if it is found to be a reflection of poor husbandry or broodstock quality then attention is required. In this case it is strongly suggested that studies be conducted to identify the occurrence of abnormalities in hatchery produced *A. japonicus*, and countermeasures be taken to minimise them if they do occur, as seed quality is a priority in stock enhancement.

Figure 2.4: Abnormalities among juvenile hatchery reared *A. japonicus* observed during current tagging study (Chapter 4). The fish on the left has a deformed upper jaw and head, while the fish on the left has an abnormally short caudal peduncle.

2.4. Conclusion

Although stock enhancement can have adverse ecological effects, with the relevant application of knowledge these effects can be controlled and maintained at an acceptable level. This could lead to a successful stocking programme, where the benefits to the fish stocks outweigh any ecological drawbacks that could not be entirely eliminated.

2.4.1. Summary of research required prior to stocking

- Estimation of size and shape of wild stock structure
- Estimation of carrying capacity of proposed release sites
    - Estimation of maximum number of fish that can be released
- Determination of competition effects on wild fish
- Selection of appropriate disease control that will not compromise genetic structure of released fish

- Comparison of seed quality between cultured and wild fish
    - Morphological study
    - Behavioural study
    - Development of methods to minimise abnormalities
- Investigation of stress responses and methods to minimise stress
- Determination of optimum release strategies
    - Optimum size at release
    - Optimum release site
    - Optimum timing of release

Management plans should cater for:

- Release of disease free fish that are no more or less susceptible to diseases than wild fish
- Release of sufficient fish to effectively enhance stocks while not exceeding the carrying capacity
- Production of seed that is morphologically and behaviourally similar to those fish in the wild
- Handling fish in such a manner as to minimise stress
- Release fish of size that display acceptable survival, while at the same time maintaining acceptable production costs.

CHAPTER 3

THE GENETIC ISSUES RELATED TO STOCK ENHANCEMENT

3.1. Introduction

The genetic consequences of stock enhancement can determine the success or failure of a stock enhancement programme. The foundation of a responsible stock enhancement programme is the understanding of a need for genetic resource management, through which the preservation of the genetic integrity of native populations can be ensured (Woodward 2000). This can only be achieved through a sound understanding of the mechanisms behind genetic influences, methods of quantifying genetic influences and scientifically backed procedures for minimising potential damaging genetic repercussion of stock enhancement.

In order to achieve the goals of a stock enhancement programme the effects of stocking must be predictable; therefore, all aspects of stock enhancement should be investigated and a management plan created to offer results, consistent with the goals of the programme. Failure to do so can lead to devastating consequences for the target stock. Negative genetic effects on recipient populations have been well documented, for example, in two cases, the stocking of *Oncorhynchus keta* and *Oncorhynchus mykiss* led to a decrease in biomass of wild stocks (Kaeriyama and Edpalina 2004, Riesenbichler *et al*. 2004). However, with an understanding of the mechanisms underlying these negative effects, stock enhancement programmes can be managed in such a way as to minimise them to an acceptable level.

This chapter discusses the need for genetic management of stock enhancement; and then reviews the methods that are available to geneticists to provide fisheries managers with the required information to produce a management protocol for a safe and effective stock enhancement programme, specific to *A. japonicus*.

3.2. A need for genetic management of stock enhancement

The reproduction of fishes, as with any other living creatures, consists essentially of two aspects:

1. Quantitative, "where the production of offspring leads to an increase in numbers" (Carvalho and Cross 1998).
2. Qualitative, "where changes in genetic structure may occur between reared fish and their wild progenitors due to differences in the proportional representation of genotypes in a population" (Carvalho and Cross 1998).

Evaluation of the effectiveness of stock enhancement programmes has traditionally focussed on the quantitative aspects of reproduction (Carvalho and Cross 1998, Serafy *et al.* 1999, Mustafa *et al.* 2003, Sanchez-Lamadrid 2004), whereby the effectiveness of the stocking programme is assessed by considering the relationship between the number of released individuals and the associated increase in fish production (Carvalho and Cross 1998). More recently, however, the importance of qualitative genetic monitoring has been recognised. Fisheries managers need a clear understanding of both the benefits and the risks involved in the stock enhancement of a fish species, and realise that if the fish is not a suitable candidate for stock enhancement, or if the enhancement programme is not managed responsibly, the artificial introduction of such fish could not only be ineffective but could have deleterious effects on the recipient population (Riesenbichler *et al*. 2004). Performance of hatchery reared fish and the effects of these fish on the recipient population can be assessed through genetic monitoring prior to, during, and following release (Carvalho and Cross 1998). A major failing in the past has been the lack of monitoring of stock enhancement programmes (Liao *et al.* 2003), the design of which should be based on well designed experimental procedures. By monitoring the effects in this way, one can obtain an understanding of whether the stocking programme is having the effect of increasing the abundance of fish or displacing wild stocks (Carvalho and

Cross 1998). In order to understand the affects of stocking, an understanding of fish stocks is required.

3.2.1. Genetic variability among individuals

The slight differences that are observed in offspring from the same parents are due to genetic variability within a species. This variation is often particularly noticeable between individuals of the same species inhabiting different confined localities (Darwin 1872). Most organisms in the wild show some form of discontinuous aggregation among individuals, both spatially and temporally (Klopper 2005). The genetic makeup of such aggregations, or stocks, is influenced by gene flow from other stocks, genetic drift and natural selection (Klopper 2005). Although it was not known by Darwin in 1872 that these variations among individuals were a function of the genetic makeup of the organisms, he understood that this variability was important for the adaptability and hence the fitness of the species.

Preservation of genetic variation within natural populations is particularly important for the preservation of ecological fitness and function, and the ability to adapt to environmental change (Jorstad *et al.* 1999), as the fitness and adaptability of an organism are largely determined by genetic factors (O'Connell and Wright 1997, Taniguchi 2003). Not only is the loss of genetic variability among individuals a matter of concern in stock enhancement scenarios, so too is the loss of variability between populations that constitute the wild stock. In wild stocks that are made up of several populations, as may be the case with *A. japonicus*, inter-population variability is important in maintaining the fitness and adaptability, and subsequent productivity, of the resource (Waples and Drake 2004).

Because of the way stock enhancement works, it has the potential to cause the replacement of locally adapted stocks with a more homogenous one from the hatchery, thereby limiting the evolutionary potential of the species in the wild (Waples and Drake 2004). Fisheries managers need to bear this in mind when developing a genetic management plan for stock enhancement. An understanding of the structure of the recipient population is required so that a captive broodstock is representative of the genetic variability that is exhibited in the wild. The various methods available to geneticists make it possible to understand the stock structure and the effects that stock enhancement may have on the recipient population. Since the understanding of the genetics related to stock enhancement is so important, tools that may provide the answers to the many questions must be investigated.

3.3. Genetic analysis methods

"Stock identification based on genetic methods allows for indirect testing of reproductive isolation and gene flow between stocks" (Klopper 2005; 11).

There are various means available to geneticists to assess the genetic structure of fish stocks, each with associated advantages and disadvantages. (Table 3.1) Polymerase chain reaction (PCR) technologies and DNA (Deoxyribonucleic Acid) sequencing provide the best method to determine genetic differences (Ward 2000). These include Microsatellite DNA analysis (MS-DNA), Mitochondrial DNA analysis (mtDNA) and allozyme analysis.

Table 3.1. Summary of the genetic tools available and their potential applications (‘xxx’ indicates the highest potential resolution; ‘-’ indicates that the marker is not appropriate at a particular level) (Bloomer 2004).

	Allozyme analysis	*Microsatellite*	*mtDNA*
Individual identity	xx	xxx	x
Relatedness	xx	xxx	x
Population genetics	xxx	xxx	xx
Stocks	xx	xxx	xxx
Between closely related species	xx	x	xxx
Species identity	xx	x	xxx
Deep phylogenetic relatedness	x	_	xxx

3.3.1. Allozyme analysis

Allozyme analysis (or protein electrophoresis) has been used since the 1970’s, and is still popular today (Klopper 2005). This method works on the principal that as enzyme proteins change in size, shape, or charge; their mobility in an electric field is affected (Chambers and MacAvoy 1999). These changes can then be detected using a dye-based chemical staining system linked to the enzyme protein’s catalytic activities. This method is an indirect indicator of genetic variation, as variation is detected in the form of amino-acid substitutions, which in turn reflect changes in the DNA (Ward 2000).

This method is a cost effective, simple and quick process, allowing for the analysis of a large sample size. However, frozen or fresh samples are required, making sample collection and storage difficult. Another disadvantage of allozyme analysis is that many species show a low level of variability at allozyme loci and some allozyme loci may be under selection (Klopper 2005), thereby producing inaccurate results.

Allozyme analysis has been shown to be ineffective in some cases for differentiating between closely related species, and separate breeding populations of the same species (Tranah *et al.* 2001, Jorgensen *et al.* 2005). Therefore, given the possible consequences of poor genetic management in a stocking programme, allozyme analysis does not seem like an appropriate tool for use in *A. japonicus* population studies to make recommendations for stock enhancement.

3.3.2. Microsatellite DNA markers

Microsatellites are short DNA segments that consist of tandem repeats of two or three base pair sequences (Mori and Higuchi 2004). Microsatellite DNA (MS-DNA) loci are codominant DNA markers that are inherited in a Mendalian fashion and are hyper-variable when compared with conventional protein markers (Taniguchi 2004). The short, tandem repeat DNA sequences that make up the microsatellites are highly variable and can provide better resolution than allozyme analysis and mtDNA for questions that are relevant to short term management (Bloomer 2004). Due to their high variability and random distribution throughout the genome, their use as markers for DNA fingerprinting has been found to be a powerful tool for the assessment of genetic divergence and pedigree analysis in broodstock management of marine fish species (Perez-Enriquez 1999, Mori and Higuchi 2004, Taniguchi 2004). Furthermore, this method allows for accurate, reproducible data that can be obtained from a large number of individuals in a relatively short time (Chambers and MacAvoy 1999), making it one of the better methods available to geneticists for population studies.

The analysis protocol for microsatellite DNA analysis needs to be developed and fine tuned for each species which takes time and money; however these investments are considered worthwhile because of the benefits of the method (Chambers and MacAvoy 1999). Once the protocol and microsatellite markers have been developed, it can be used over and over for that particular species, and the DNA elements can be amplified by polymerase chain analysis and analysed by electrophoresis, producing data that can easily be analysed (in much the same way as allozyme analysis) (Chambers and MacAvoy 1999).

Our understanding of mechanisms of evolution of microsatellites are however unclear (Burg *et al.* 1999, Chambers and MacAvoy 1999), with a consequent inability to produce entirely satisfactory models for allele frequency spectra in populations (Chambers and MacAvoy 1999). However the current popularity of the method suggests that most analysts do not regard this as a major problem (Chambers and MacAvoy 1999).

The use of MS-DNA markers has become the most widely used DNA technology (Li *et al.* 2004), and has been used for examining the genetic variability of wild, broodstock, and offspring samples monitoring changes in genetic variation of farmed stocks, parentage assignment, and fine scale studies of population structure, of a number of fish species.

MS-DNA assessment can be used to:

- Assess the genetic variation of fish populations (Tranah *et al.* 2001, Jorgensen *et al.* 2005)
    - By surveying the mean number of alleles and heterozygosities
- Evaluate the effective population size (Perez-Enriquez 1999, Sekino *et al.* 2003, Taniguchi 2004).
    - Genetic variability decreases as the effective population size decreases
    - MS-DNA markers are effective at estimating heterozygosities, and N_e is estimated from the number of heterozygosities (H_e) in the following formula: $N_e = (H_e / (1 - H)) / 4\mu$
      Where $\mu = 4 \times 10^{-4}$ (mutation rate for MS-DNA) (Taniguchi 2004).
- Estimate the inbreeding coefficient (Perez-Enriquez *et al.* 1999, Sekino *et al.* 2003, Taniguchi 2004)
    - There is low genetic variability when inbreeding occurs
    - The inbreeding coefficient (F) can be calculated from the effective population size (N_e): $\Delta F = ½ N_e$ (Taniguchi 2004).
- Determine the amount of change in genetic variation from broodstock to offspring generations (Perez-Enriquez 1999).

3.3.2.1. Evaluating effective population size:

The effective population size (Ne) can be estimated by the number of heterozygosities observed and by taking into account the mutation rate of MS-DNA (Taniguchi 2004). As Ne becomes smaller, there is a higher degree of inbreeding within the population and a subsequent reduction in genetic variability.

In studies by both Tranah *et al*. (2001) and Jorgensen *et al.* (2005) on the population genetics of sturgeon and herring respectively, allozyme analysis was unable to reveal relevant differentiation, and in the case of the sturgeon, was not able to distinguish between the two species in the study, while microsatellite DNA analysis showed significant differences in both cases. This illustrates that MS-DNA analysis is a more powerful method for distinguishing between fish stocks.

3.3.2.2. Estimate the inbreeding coefficient:

Because the use of a relatively small broodstock numbers in stock enhancement, the genetic variability of the wild stock can be reduced, causing a loss of fitness in the population and an increase in the occurrence of inbreeding (Perez-Enriquez *et al.* 1999). Thus, knowledge of both the population size and the inbreeding coefficient are of great importance.

3.3.3. Mitochondrial DNA analysis

Mitochondrial DNA is inherited maternally, and does not undergo recombination, therefore remaining unchanged from one generation to the next, unless mutations occur (Mori and Higuchi 2004). mtDNA is a useful tool for molecular analysis because it is made up of genes that evolve faster than most nuclear genes (Seeb *et al.* 1998, Burg *et al.* 1999). These fast evolving regions are useful for studies at the population level (Burg *et al.* 1999), as changes can be detected over shorter periods of time.

Mitochondrial DNA analysis has been successfully used to detect changes in population structure in a number of marine fish species (Klopper 2005). However, the use of mtDNA can be limited in cases where high levels of genetic diversity occur and in other cases may not be sufficiently sensitive to provide conclusive results. The usefulness of mtDNA as a tool for analysing genetic diversity is questionable, due to high costs and slow throughput (Seeb *et al.* 1998). Furthermore, because it does not recombine and is maternally inherited as a single locus, mtDNA data occasionally reveals less diversity than allozyme analysis so that variation is linked entirely with the geographic resolution apparent for similar populations (Seeb *et al.* 1998). However, as mtDNA not only accumulates mutations fast, but also showed that deleterious mutations may be fixed due to the relaxed control over the DNA repair and replication, it should still be regarded as a useful tool and should be used in conjunction with nuclear DNA, as they can provide complementary information (Seeb *et al.* 1998).

3.3.4. Suggested methods for use in genetic management for stock enhancement of *A. japonicus*

Although allozyme analysis has been the most widely used method, MS-DNA analysis is becoming more popular and is an ideal method for fine scale stock structure investigations (Klopper 2005), such as those required prior to stock enhancement of *A. japonicus*. Klopper (2005) suggests that MS-DNA analysis be used to investigate the structure of *A. japonicus* stocks is South Africa.

Bloomer (2004) and Klopper (2005) recommend the use of more than one genetic marker (preferably all three) when conducting a genetic studies of fish stocks in order to attain a full understanding of the processes that shape species and their populations. However, it appears that MS-DNA would be the best choice of methods for determining the effective population size as well as determining the inbreeding coefficient in a hatchery as well as in the wild population subsequent to the commencement of stocking. MS-DNA markers have been used to estimate genetic variability, effective population size and inbreeding coefficient for management decisions for the stock enhancement of *Pagrus major* in Japan (Taniguchi 2004). Focus should also be placed on long term studies to detect any chances in structure of wild stock as a result of stock enhancement.

3.4. Application of genetic methods to stock enhancement

In the 1980's concerns were raised regarding the effects that hatchery-reared fish could have on the genetic variability of wild populations (Kitada and Kishino 2004; Taniguchi 2003). There are three major concerns. Firstly the transfer of genetic material from the limited broodstock gene pool coupled with unnaturally high survival among larval and early juvenile stages in the hatchery (Waples and Drake 2004) may result in genetic bottlenecks, whereby the genetic variability present in the wild stock could be lost (Blanco *et al.* 1998, Taniguchi 2003). Genetic bottlenecks are caused by inbreeding within a confined population as a result of low genetic variability. The second major concern is the founder effect, which is the introduction of new genetic makeup that may be less resilient to environmental stressors or biological change. Traditionally, in an aquaculture situation, intentional genetic improvement of the cultured fish is carried out, through selection, cross breeding or hybridization, which has led to the third major concern in aquaculture facilities of the escape of hatchery reared fish into the wild and the subsequent mixing with wild population causing reduced fitness.

Until recently, assessment of this genetic dilemma remained qualitative as there was no way to quantitatively evaluate the severity of the effects on the wild population. However, methods to evaluate the severity of such genetic concerns have since been developed and are described by Kitada and Kishino (2004). Kitada and Kishino (2004) use an empirical Bayesian procedure for estimating genetic differentiation among populations. The results of releasing hatchery reared fish into the wild may have unpredictable effects on the natural population, ranging from no detectable introgression into indigenous populations to a complete displacement of the natural population (Blanco 1998). Using mtDNA methods, genetic differences have been reported between hatchery reared fish and wild individuals for several species, including black sea bream *Acanthopagrus schlegeli*, (Taniguchi *et al.* 1983, cited in Kitada and Kishino 2004), red sea bream *Pagrus major* (Tabata *et al.* 1997, cited in Kitada and Kishino 2004), and Japanese flounder *Paralichthys olivaceus* (Fujii & Nishida 1997, Sekino *et al.* 2002, cited in Kitada and Kishino 2004). It is therefore important that a long-term monitoring strategy be implemented to assess and document any differences between *A. japonicus* in the hatchery and in the wild as well as any changes in genetic structure of the enhanced stocks that may have occurred. This should be done by continually conducting genetic studies on the enhanced stock, using the genetic methods discussed.

Long term monitoring of stock structure is especially important if a restocking programme continues for several generations. In such cases the risk of inbreeding in the wild increases, as a large proportion of the fish would have been produced in the hatchery and all of these are likely to be from the same broodstock (Waples and Drake 2004). It is necessary to be able to identify whether there is a genetic difference between the hatchery-reared fish and the wild population, and whether this difference is sufficient to pose a threat (Kitada and Kishino 2004). Such studies have been conducted on different fish species with mixed results (Kitada and Kishino 2004). A similar study is necessary for *A. japonicus*. Careful evaluation of the genetic variability of the stocks, using the methods described, is necessary to determine an appropriate hatchery broodstock size that will minimize potential genetic problems (Gold 2004).

3.5. Genetics of *A. japonicus*

Marine species, such as *A. japonicus*, occur in a largely homogenous environment with few physical barriers to limit the flow of genes (Cross 1999). The result is that, marine species (migratory species in particular) typically show little variation between populations. Several studies have shown that a high dispersal potential, due to large population size, a migratory adult life history strategy, or larval dispersal often results in a low level of genetic differentiation within the species (Klopper 2005). Until recently, the genetics of *A. japonicus* had not been studied, and commercial catch returns and tagging data pointed towards a single stock in which genetic differences were thought to be unlikely (Griffiths 1995). However, increasingly, studies have shown that this may not be the case, and that behavioural philopatry at various life stages may be responsible for limited gene flow and population differences (Klopper 2005), therefore, one cannot make assumptions about the population structure of a species based on an incomplete understanding of its life history, as is the case with *A. japonicus*. It is important that the genetics of new candidate species for stock enhancement should be studied and understood prior to stocking. A study by Klopper (2005) on the intraspecific genetic variation of *A. japonicus* found evidence of isolation-by-distance within the stock, suggesting that the stock may not consist of one freely intermixing unit but rather geographically separate spawning populations. However, due to the small sample size (n=133), the assortment of samples from various cohorts, and a lack of power in the population analysis tests, this evidence is not conclusive and a more comprehensive study is required (Klopper 2005).

3.5.1. Understanding the genetic structure of Argyrosomus japonicus stocks

The most commonly used definition for a stock is "an intraspecific group of randomly mating individuals with temporal and special integrity" (Ihssen *et al.* 1981). Because there is little or no flow of genetic material between stocks that are spatially isolated (as they are not randomly mating), then genetic differences start to develop between the stocks (similar in process to speciation, but on a smaller scale). Such differences may be detected by allozyme, mtDNA or microsatellite analysis.

Acquisition of data that would provide an understanding of the population structure of *A. japonicus* would necessitate collection of genetic material from fish throughout the distributional range of the species. The samples collected for this study would need to include a record of location (including depth and distance offshore), date and the length of the fish. Special attention should be paid to the collection of samples from spawning aggregations as this could provide valuable information (Klopper 2005). These samples could then be analysed using the appropriate method and would indicate whether there is evidence of genetic variability within the South African *A. japonicus* population. The results of which would illustrate whether the species exists as a single stock or if there are signs of isolation by distance, which would, as mentioned, have implications for a stock enhancement programme.

It has been mentioned that genetic analysis methods can be used to detect signs of limited genetic mixing between populations. However, it should remain clear that genetic studies should not be used exclusively. There are complex interactions among physical environmental factors, life history characteristics and population genetic variability and differentiation within marine taxa, which must be taken into account when addressing stock level questions using genetic techniques (Bloomer 2004).

As with many marine species, *A. japonicus* has an enormous reproductive potential, with a single female capable of producing up to 2 million eggs in a single spawn. This matches the hypothesis proposed by Hedgecock (1994, cited in Gold 2004) whereby a small number of individuals could replenish entire populations, if mating coincides with favourable environmental conditions, resulting in high survival among the large number of offspring (Gold 2004). This is similar to what would happen in a stock enhancement situation, and may be the case with red drum, *Sciaenops ocellatus*, where there is known to be a large variance in the offspring from a single parent. Where there is a low N_e to population size ratio fish also exhibit enormous reproductive potential (Gold 2004). Occurrence of this in wild *A. japonicus* stocks (creating a low natural N_e value) suggests that this species may be a good candidate for stock enhancement, as the N_e value in the hatchery would not be very much lower than that in the wild, meaning that genetic effects may be less severe. However there are no data to support this for *A. japonicus* and this requires further investigation. The data required include knowledge of the effective broodstock size, which would involve genetic analysis, as well as an estimate of the sex ratio for the species (Gold 2004).

3.6. Hatchery Management

The ability to investigate the genetic structure of fish stocks and detect and quantify the genetic effects of stock enhancement are the only the first step towards creating a management protocol to reduce any genetic ill-effects that may be attributed to stock enhancement. There are several important considerations that should be taken and practices that can minimise the genetic effects of stock enhancement.

3.6.1. Broodstock collection

The broodstock management protocol employed within a hatchery, whether for the purpose of commercial aquaculture or stock enhancement, should be a function of the goals of that particular hatchery. Therefore in the case of a commercial aquaculture facility, broodstock management may be in favour of producing fast growing, disease resistant, non-aggressive, good tasting, or good looking fish. On the other hand, broodstock management for stock enhancement would select a pool of fish that are a realistic representation of the recipient population.

The selection of suitable broodstock for a stock enhancement programme is perhaps one of the most important steps towards a responsible management protocol. To have a broodstock that is representative of the recipient population, fisheries managers must have a thorough understanding of the genetic structure and population biology of the recipient population, which can be gained through the use of MS-DNA analysis methods. Broodstock should be collected from the local population where the offspring are to be released (Taniguchi 2004) if possible, and should represent all year classes of sexually mature fish of the species and from different life history strategies, that occur within the population (Cross 1999), as some life history parameters are genetically determined (Hauser *et al.* 1998). In *A. japonicus* these different life history strategies are exhibited by those individuals that undergo different spawning migrations, with some fish spawning in the Southern Cape, some in the Eastern Cape and some in KwaZulu-Natal (Klopper 2005). The use of family groups as broodstock should be avoided, as this will result in inbreeding, which reduces genetic variation and increases genetic drift.

It may be assumed that a random selection of broodstock from the wild would produce a representative sample. However, by chance, some types will be overrepresented while others are underrepresented (Waples and Drake 2004). This risk can be decreased by taking a larger sample size (Waples and Drake 2004). A further concern is that certain traits may be well represented in the broodstock while others are not (Waples and Drake 2004). In order to avoid this, genetic studies should be performed on a large number of potential broodstock fish, from which a suitably representative sample should be selected.

Large effective broodstock numbers (N_e) are important to minimize the loss of genetic variability. The N_e is "a theoretical expression, which is equal to the actual number of parents when the numbers of males and females are equal, but reduces quickly as the sex ratio varies from equality" (Cross 1999). As the N_e value is a theoretical value based on an idealized population and almost no natural population is ideal (Waples and Drake 2004), the N_e is usually smaller than the number of breeding individuals in the wild. To calculate the required N_e for stock enhancement one needs to know the N_e for the wild population. Although without this available data an N_e value of >50 individuals is essential but a value of >100 individuals is recommended by Cross (1999) as a broodstock N_e. However these values are a guideline and can be subjective and highly varied. The Alaskan Department of Fish and Game's, "finfish genetic policy for Alaska" (Moore and Seeb) insists on an N_e value of at least 400 fish. It is therefore suggested that investigation be done into the required N_e value for *A. japonicus*. As discussed in the review of available genetic methods, MS-DNA analysis is a suitable tool for measuring the effective population size of the wild population as well as the potential broodstock.

Outbreeding depression is another possible problem that should be taken into account when considering releasing hatchery reared fish into the wild for stock enhancement purposes. This is a reduction in fitness resulting from the crossing of individuals from genetically divergent populations (Waples and Drake 2004). Loss in fitness can be due to a loss of local adaptation or from a breakdown of coadapted genes (Waples and Drake 2004). Loss of local adaptation occurs by mixing fish that are locally adapted to an area with fish from a non-local population resulting in fish that are less adapted to the area. This only occurs in species that have localised populations through their distribution.

3.6.2. Broodstock selection and management

The selection of appropriate broodstock is one of the most important steps towards minimizing the genetic risks associated with stock enhancement. One needs to consider several factors when selecting broodstock. The population structure of the wild population should be understood. This would provide an indication of where to collect broodstock from, for stocking certain areas (if there is any stock separation among the wild population). Each of the potential broodstock fish should be genetically examined to ensure that those fish used as broodstock are representative of the genetic diversity present in the wild population. Furthermore, once the stocking programme is operational, it should be ensured that new broodstock collected should not be a hatchery reared and released fish. The use of offspring from hatchery reared fish should be avoided, however, in the case of *A. japonicus*, which reaches maturity at about 6 years (Griffiths 1996), this would only be a possible problem after 12 years of stock enhancement.

The use of microsatellite DNA markers has been successful in the estimation of genetic diversity, effective population size and inbreeding coefficient.

Once the genetic structure of the population is known, potential broodstock should be collected and the genetics of these fish analysed to ensure that a broodstock representative of the wild population is used in the stock enhancement programme. Genetic material collected from the potential broodstock fish and analysed using the suggested methods discussed would be compared to that of the wild population.

3.6.3. Spawning

A variety of methods are used by hatchery managers for spawning fish in captivity. Certain fish species are strip spawned and the eggs and sperm are mixed together in a bowl, in which fertilization takes place. Other species cannot be effectively strip spawned but can be induced to spawn spontaneously after injection with hormones, or if they are subjected to specific temperature and light conditions. *A. japonicus* can be induced to spawn spontaneously by means of hormones and pellet implants (Primary Industries and Resources SA 2001) or by subjecting them to specific temperature and light conditions, alternatively, strip spawning methods can be used. By using hand stripping methods the N_e can be accurately controlled. To prevent loss of genetic variability, sperm from different males should be kept separate before mixing with the eggs, not all males sperm will contribute equally to fertilization, and the effective N_e value will be decreased (Cross 1999). In species that are not strip spawned, the effective N_e value can be lowered considerably as certain individuals may not spawn, and others may spawn numerous times. When inducing spontaneous spawning, molecular monitoring should be used to assess the contribution from each of the broodstock individuals (Cross 1999), and, if found to be unsatisfactory, measures should be taken to rectify the problem.

The number of hatchery reared fish that are released into the wild has a direct influence on the severity of any genetic problems that may occur. For the purpose of stock enhancement, the primary goal of which is to increase the natural population size, releasing a certain minimum number of fish is important. Releasing too few fish will not effect an increase in numbers. Conversely, however, by releasing too many fish and creating a high ratio of hatchery released fish to wild conspecifics, genetic effects of hatchery rearing may be compounded (Waples and Drake 2004). It is therefore necessary to determine the actual number of fish in the wild population (ie absolute abundance). There is therefore a balance between stocking too few fish and having an ineffective stocking programme and too many fish and having detrimental effects on the population.

3.6.4. Selection

As has been mentioned, in hatcheries that produce fish for aquaculture, whether for food or for ornamental purposes, a selection process is undertaken to select for favourable traits in the fish. By selecting for certain traits, the genetic variability amongst the offspring is reduced. This selection does not only occur artificially by purposefully removing unwanted fish but also passively whereby fish undergo "natural selection" under the artificial hatchery conditions, including husbandry methods and unnatural light, temperature, stocking densities and water conditions, which can all affect survival among juveniles in such a way that certain traits are favoured (Cross 1999). This is known as domestication and has been documented in several hatchery reared species including *Oncorhynchus mykiss* (Riesenbichler 2004). Conditions in a stock enhancement hatchery should be kept as close as possible to those in the juvenile fish's natural environment, in order to reduce domestication of hatchery reared fish. These effects cannot be entirely eliminated as there would be no survival advantage in the hatchery over natural conditions. If conditions were kept identical there would be no point to having the hatchery (Waples and Drake). The release of "domesticated" fish into the wild can lead to problems, not only in the first generation released into the wild, but also in the progeny of these released fish that interbreed with the wild population. The selected traits in the process of domestication may include physical as well as behavioural attributes, both of which can affect the fitness and survival of these fish and their offspring. This can, however, be minimised by the early release of juvenile fish.

A further area of concern in the release of hatchery reared fish, which has been little researched, is the effect of the unnaturally high survival rates within the hatchery, on the wild recipient population. In the wild natural selection eliminates the weaker individuals and thereby maintains the fitness of the population (Darwin 1872). It is possible that high survival in the hatchery as a result of maintaining the conditions favourable for survival may result in a decrease in the natural selection effect, with hatchery reared fish being consequently less fit on average than their wild conspecifics. Fitness within the wild population may therefore be reduced by interbreeding with such weaker hatchery reared fish. This problem is emphasized by the concern regarding the effects of possible escape or release of genetically managed aquaculture fish into the wild, which may result in introgressive hybridization or other ecological implications (Lam, online).

Where release is envisaged, individuals should be bred differently to those for captive culture, with focus on minimum genetic change and maximum representation of the wild population, as opposed to desirable culture traits (Lam, online). These effects can be reduced by the use of semi-natural rearing conditions, so that some natural selection does occur. However, this is not common under hatchery conditions (Cross 1999). This area problem deserves further research prior to the release of hatchery reared *A. japonicus* into the wild.

3.7. Summary of required research prior to stocking

Knowledge necessary for fisheries managers to create a responsible protocol, in terms of the genetic effects, for the stocking of juvenile *A. japonicus* in South Africa includes:

1. An understanding of the genetic structure of wild *A. japonicus* stocks using a combination of both MS-DNA and mtDNA analysis methods.
   - To ascertain whether there are separate populations of *A. japonicus* along South Africa's coast line.
   - To determine where broodstock should be collected from and where juveniles should be released.
   - For comparison of wild stock and potential broodstock to assess the suitability of these individuals as broodstock.

2. Estimate of population size and effective broodstock size of *A. japonicus* using a combination of both MS-DNA and mtDNA analysis methods.
   - To help estimate the severity of genetic effects of releasing hatchery reared fish.
   - To determine the required broodstock size in the hatchery.

3. Assessment of the potential effects of hatchery conditions on the genetics of hatchery reared *A. japonicus*.
   - To understand what possible risks, specific to *A. japonicus*, are presented by releasing hatchery reared juveniles into the wild.
   - To develop hatchery management techniques that minimise genetic changes from wild *A. japonicus*.

4. Assessment of potential effects of hatchery reared fish on genetics of wild fish.
   - To determine what the "acceptable" levels of genetic differences, between hatchery reared fish and wild fish may be.
   - Predict the effectiveness of the stock enhancement programme.

3.8. Application of genetic methods

Depending on the appropriateness of each of the methods for the use on *A. japonicus*, the chosen method or combination of methods would be used to test for evidence of genetic variation between individuals or sub-populations. This would be able to provide information for all of the required studies: Understanding the structure of the stock, effects of hatchery conditions on genetics of hatchery reared fish, and effects of hatchery reared fish on wild fish. The sampling protocol would necessarily be designed in such a way that the genetic analysis would provide the relevant information.

3.8.1. Evaluating the effects of hatchery conditions on the genetics of hatchery reared *A. japonicus*:

This study would involve collecting genetic material from hatchery-reared *A. japonicus* (reared under similar conditions to those for stock enhancement), and once a programme has been initiated, from those fish intended for release. These samples should then be compared with those from the wild population to determine whether selection pressure within the hatchery is altering the genetic variability in the fish produced in it.

3.8.2. Genetic monitoring

During a stock enhancement programme there must be a genetic monitoring system in place to detect genetic effects that may be occurring as a result of the stocking. Taniguchi (2004) has advised five steps that should be taken towards a genetic monitoring programme.

1. Develop MS-DNA markers for examining genetic divergence of local populations in the wild.
2. Evaluate genetic variability and estimate H_e, N_e, and F values of founders, captive broodstock, and seed fish for release.
3. Make a plan of spawning and crossbreeding for maintaining the genetic variability and prevent inbreeding based on the genetic parameters: H_e, N_e, F, and $\hat{r}$ (relatedness among individuals).

4. Monitor the indicators of genetic variability such as H_e, N_e and F values of descendants over a number of generations.
5. As a last resort, conduct selective breeding based on the minimal-kinship broodstock management to maintain the genetic variation and to prevent accumulation of inbreeders.

3.9. Conclusion

As illustrated, there are numerous risks associated with the stocking of hatchery reared fish into the wild. However, with the correct information and hatchery management procedures and practices, these risks can be minimised to an acceptable level. There are many areas which have been identified in this chapter, specific to *A. japonicus* that require investigation, prior to the implementation of a stocking programme. These investigations are necessary and worthwhile as there are substantial benefits that could be gained from the stocking of *A. japonicus* in South Africa. If fisheries managers integrate the available genetic management protocols into any proposed stock enhancement programme, the genetic profile of the wild population can be monitored and maintained.

CHAPTER 4

AN EVALUATION OF TAGGING METHODS FOR JUVENILE *ARGYROSOMUS JAPONICUS* FOR POST RELEASE MONITORING OF STOCK ENHANCEMENT

"The identification of fish over time, either as a individuals or as a member of a group, is one of the basic requirements of studies related to ecology, biology, population dynamics, or fisheries management" (Buckley & Blankenship 1990, 173).

4.1. Introduction

In Blankenship and Leber's (1995) "Responsible approach to marine stock enhancement", the importance of determining and implementing means to identify hatchery produced fish is highlighted. The ability to identify hatchery reared fish allows for the estimation of survival, growth rates and contributions to the wild population and the fishery (Taylor *et al*. 2005b), and can therefore be used to assess the effectiveness of the stocking programme. Post release monitoring of a stock enhancement programme is essential in evaluating both environmental effects and economic success or failure. Post release monitoring is important so that in the case of an unsuccessful programme, efforts can be ceased immediately in order to prevent possible further financial loss or damage to the environment.

The effects of stocking should not be monitored using traditional stock assessment methods, as they can easily be masked by natural fluctuations in the wild population, leading to inaccurate assessment of the effectiveness of the programme. In the past, numerous stock enhancement programmes were run without quantification of their success, thus, confidence in their effectiveness was lost (Molony *et al*. 2003). For several decades little attention was paid to stock enhancement, until the advent of, and rapid technological advances in modern tagging technology, making it possible for fisheries managers to monitor the effects of stocking more accurately.

Although distinction between hatchery produced fish and wild fish subsequent to release may appear simple, has been one of the principal challenges associated with stock enhancement (Woodward 2000). This is because the “ideal” fish tag for small fish in a stock enhancement scenario does not exist. Characteristics of such a tag would include ease of application to large numbers of small fish at a relatively low cost, high retention rates over long periods, ease of detection by scientists as well as anglers and no interference with life processes of the tagged fish. In recent, the development of new, improved tagging methods has given scientists more options for post release monitoring of hatchery produced fish (Woodward 2000).

The purpose of this study, therefore, was to identify a suitable tagging method for use in post release monitoring of *A. japonicus* stock enhancement, as well as a tagging method suitable for use in pilot studies investigating release strategy options and ecological impacts of release. The tagging method for long-term post release monitoring will require qualities such as ease of application, low cost, and long-term tag retention, whereas that for use in shorter term pilot studies is not required to be as inexpensive or easy to apply, and is not required to exhibit retention over long periods of time, as pilot studies are likely to be conducted on smaller special and temporal scale of perhaps 2 to 3 years (Refer to Chapter 3).

<u>4.1.1. Pilot study</u>

Pilot studies, necessary prior to stock enhancement, should assess factors such as appropriate release strategies (number of fish, size at release, location, time of release, etc.), ecological impacts and initial potential effectiveness of stock enhancement of *A. japonicus* (Refer to Chapter 3). Although the tagging method for the pilot study need not be the most cost effective or least time consuming, it must provide acceptable survival rates and tag retention over the full duration of the study. The suitability of the tagging method for the pilot study will be highly dependant on its ease of detection. As tag loss and tag associated mortality usually occur during the time soon after tagging, appropriate methods can be identified in a relatively short period (±5 months).

4.1.2. Stock Enhancement

Post release monitoring of hatchery reared fish is used to determine the effectiveness of the stocking programme as well as to quantify its effect on the ecosystem. All fish that are released from the hatchery during the stocking programme should be tagged in such a way that that they can be identified as hatchery-reared fish at any stage after release. This would necessarily be an ongoing study that should continue even after the cessation of the stocking programme. Post release monitoring in stock enhancement therefore requires a tag that offers high survival rates and long-term tag retention that should in no way compromise the health, survival, or behaviour of the tagged fish. It also requires a tag that is inexpensive and that can be easily and quickly applied to a large number of fish (Taylor *et al*. 2005a).

4.2. History of tagging

The tagging of fish dates back as far as the 17^{th} century, where individuals marked juvenile Atlantic salmon (*Salmo salar*) by tying ribbons to their tails, to demonstrate their ability to return from the sea to their natal river (McFarlane *et al.* 1990). Technology has since improved, as have the tags, giving scientists the ability to tag large numbers of small fish quickly, with tags that do not affect any life processes of the fish. While tags were originally mostly external type tags, popularity of the use of internal tags, particularly for small fish, has grown.

External tags range from attachment type tags such as threads, wires, plates, disks, rings, bands and straps, to mutilation, pigments or stains and brands (McFarlane *et al.* 1990). However, most external tags tend to be relatively large and poorly suited to small fish, suggesting that internal tags may be more suitable for use in stock enhancement programmes.

Internal tags include those that are externally visible, those that require a detector of some sort to distinguish between tagged and untagged fish, and those that are internally detectable. Externally visible internal tags have evolved over time from the use of tattoo-ink (Laufle *et al.* 1990) and fluorescent grit (Nielson 1990), to the more recently available visible implant fluorescent elastomer (VIFE) tag[1]. Internal tags that require detectors include Passive Internal Transmitter (PIT) tags, Coded Wire Tags (CWT) and acoustic tags, while internally detectable tags include fluorescent dyes such as alizarins and Oxytetracycline.

4.3. Available Tagging Methods

Fishes may be tagged for numerous reasons in research as well as in commercial aquaculture and stock enhancement. A number of methods are available for tagging fishes for the purpose of scientific research, and in commercial aquaculture; however many of the tagging methods are only suitable for certain size classes of fish, are limited to use in certain species, or are too costly or time consuming for mass tagging such as that required for stock enhancement. Table 4.1 shows the suitability and limitations of a number of these tagging methods.

[1] Northwest Marine Technology Inc. Fish marking and tagging systems. www.nmt.us

Table 4.1: Suitability and limitations of selected tagging methods.

Tag type	Minimum size of fish that can be tagged	Cost	Suitable for juvenile *A. japonicus*	Suitable for use in stock enhancement
Anchor tag			No	No
Dart tag	25 cm TL (Paul Cowley, pers comm)	R 4 per tag (Paul Cowley, pers comm. September 2005)	No	No
CWT	5 cm TL	± R 0.70 per tag (Northwest Marine Technology Inc. 2006)	Yes	Yes
VIFE tag	50 mm TL (Doupé *et al.* 2003)	± R 0.80 per fish (Northwest Marine Technology Inc. 2006)	Yes	Yes
Passive Internal Transmitters (PIT tag)		R 20 per tag (Paul Cowley, pers comm. September 2005)	No	No
Alizarin Complexone	No limitation if submersion is used	R 75-R 300 per 100,000 fish (Taylor *et al.*2005a)	Yes	Yes
Oxytetracycline-HCL (OTC)	No limitation if submersion is used	R 75 per 100,000 fish (Taylor *et al.* 2005a)	Yes	Yes
Acoustic tag		R 2000 per tag (Paul Cowley, pers comm. September 2005)	No	No

4.3.1. Unsuitable tags for stock enhancement

Certain tags are not suitable for use in a stock enhancement scenario, either due to their large size, cost, or the time consuming or difficult nature of their application, for example anchor or dart tags due to their large size. Owing to their large size, they are more suited for use in larger individuals and can compromise the survival or welfare of smaller or juvenile fish by altering aspects of their behaviour (Doupé *et al*. 2003), as well as the possible incidental mortality associated with the application process. Furthermore, these tags would incur excessive cost and time required to tag large numbers of individuals, such as would be required for stock enhancement purposes. These tags, therefore, do not meet the requirements of tagging methods for post release monitoring in a stock enhancement programme.

Similarly, the high cost (R 2000 each), large size and the time required for surgery, suggest that acoustic tags are not suitable for use in stock enhancement programmes. Although physical retention of tags is high, the effective detectability of these tags is limited to the battery life of the tag, a maximum of a few months (insufficient even for a pilot study). Acoustic tags are intended especially for the tracking of fish for experiments involving the tracking of fish movement and were not designed for use in this type of study.

Passive Internal Transmitter (PIT) tags are similar to coded wire tags in that they are inserted into the fish and are detected magnetically rather than visually. However, PIT tags are much larger (12 mm long) and therefore less suitable for use in small fish than the smaller coded wire tags (1.1 mm) (Northwest Marine Technology, Inc. 2000). Coded wire tags and PIT tags share some of the same advantages and disadvantages; however the suitability of PIT tags for use in smaller fish (as is required in a stock enhancement programme) is reduced by their larger size. Coded wire tags are therefore more suitable in terms of size and will be tested as one of the potential methods of tagging hatchery reared juvenile *A. japonicus* for both the pilot study and the actual stocking programme.

The tags that were evaluated in this study were selected on the basis of their suitability for use in small fish, their cost effectiveness, and the ability to be used for large numbers of individuals. Based on these criteria only CWT, VIFE and OTC were determined suitable for stock enhancement, and were thus compared in a tag retention study to determine the most suitable tagging method for a pilot study and for the long term monitoring programme.

4.3.2. Coded Wire Tags (CWT)

The coded wire tag was developed in the 1960's (Jefferts, 1963) for studies on salmon migration, and this remains their dominant application today. Coded wire tags have been tested and used for management and research purposes on over 27 fish genera (Buckley & Blankenship 1990), including *Sciaenops ocellatus* from the family *Sciaenidae* (the same family as *A. japonicus*) and have been used successfully on fish as small as 22 mm TL (Solomon 2005).

CWT's are made of stainless steel wire of 0.25 mm diameter and have a length of 1.1 mm (Northwest Marine Technology, Inc. 2000). Half length, one and a half and double length tags are also available for use in fish of various sizes. Tagged fish are identified by detecting the tag with a specially designed metal detector produced by Northwest Marine Technology, Inc. The coded wire tags themselves are fairly inexpensive, however, setup costs of automated applicators and detectors are high (Taylor *et al.* 2005a, Northwest Marine Technology, Inc. 2006). CWT's also have a factory etched decimal code which allows for identification of fish by batch or of individuals. This could be extremely useful in the monitoring of different cohorts, release sites, or even fish from particular parents. If found to be suitable, in terms of retention and survival, CWT's could prove to be particularly useful, and the initial setup costs may be worth while.

The coded wire tag is implanted into the cartilage, musculature, or connective tissues of the fish, using a hypodermic needle by manual or automated techniques. If done correctly, this has minimal impact on the survival, growth or behaviour of the fish (Solomon 2005). However, if tags are implanted incorrectly or in an unsuitable location on the fish, they can cause significant problems to the fish (Buckley & Blankenship 1990) or may even be lost. Therefore, fishes tagged correctly should show little or no tag related mortality. Additionally, large scale programmes, involving coded wire tagging of juvenile salmon have shown coded wire tags to exhibit acceptable retention rates, with an average tag loss of 5% after 30 days and no significant increase in tag loss after 200-300 days (Blankenship 1990). However tag retention can vary according to the skill level of the person applying the tags, thereby altering results.

Histological examinations by Bergman *et al.* (1968) have shown that the stainless steel wire, of which the tags are made, has minimal interaction with the tissues in the fish, which could otherwise affect both survival and retention of the tag. Survival and retention studies using coded wire tags have shown variable results (Table 4.2).

Table 4.2: Tag retention and survival of various fish tagged with coded wire tags.

Fish	Location	Retention	Survival	Reference
Oncorhynchus tshawytscha & *O. kisutch*	USA & Canada	> 95%		Blankenship 1990
Lates calcarifer	Australia	> 96%	> 99%	Rimmer and Russell 1998
Polydactylus sexfilis	Hawaii	> 93%		Friedlander and Ziemann 2002
Sciaenops ocellatus	USA	poor	low	McEachron *et al.* 1998

McEachron *et al.* (1998) showed that survival and tag retention in *Sciaenops ocellatus* are poor, and suggests that this method is not suitable for use in a stock enhancement programme. However, CWTs are currently being used on *Sciaenops ocellatus* for post release monitoring of stock enhancement in Tampa Bay, Florida (USA) (Dodd 2003). Coded wire tags have not been tested on *A. japonicus* and their low cost and small size suggest that this method is, at least, worthwhile testing if only for application in pilot studies.

4.3.3. Oxytetracycline-Hydrochloride (OTC)

Oxytetracycline hydrochloride ($C_{22}H_{24}N_2O_9$) is a drug, approved by the US Food and Drug Administration (Meinertz *et al.* 2001), which is commonly used as an antibiotic for humans and animals including fish. OTC is also a commonly used fluorescent dye for tagging fish. The chemical structure of teleost otoliths is such that, when exposed to OTC, the OTC becomes incorporated into the growing calcified tissue, such as the calcium carbonate matrix of the otolith (Pollard *et al*. 1998), allowing for later identification. The OTC incorporated in the otolith produces a characteristic fluorescent emission when illuminated with UV light (Brothers 1990) allowing scientists to distinguish between hatchery and wild fish. OTC is used extensively in age and growth studies but is also extremely useful for batch marking fish for later identification. Methods for marking the fish include injection of OTC into the fish (commonly used in larger fish), immersion in an OTC solution, or incorporation into the fish's diet (Brothers 1990).

Tag retention studies have not conclusively shown how long the stain is retained in the otolith but have shown that it is still present in excess of three years after tagging in *A. japonicus* (Taylor *et al.* 2005a). It has also been suggested that OTC remains in the otoliths of a tagged fish indefinitely (Dr. A.J. Booth, Dept. Ichthyology and Fisheries Science, Rhodes University, pers comm. March 2006).

The advantages of OTC are that it is inexpensive and can be easily and quickly applied to batches of fish. With immersion methods available to batch mark fish, one can mark thousands of fish in a single group without having to handle each fish individually. This requires less time and exposes the fish to less stress than other tagging methods, where fish may be handled individually, such as with coded wire tags.

As shown in Table 4.4 the disadvantage of using OTC is that fish need to be sacrificed in order to remove otoliths for the detection of OTC under a microscope using UV light. This also makes detection of tagged fish time consuming and impossible in the field. Although otoliths offer the best results in terms of tag identification for OTC (Taylor *et* *al.* 2005a), fin spines can also be examined under UV to detect OTC. This provides a suitable non lethal method for detection of tags and hence identification of hatchery reared fish. However, in the case of stock enhancement, fish that enter the fishery and are captured can be examined and their origin determined using this method as they are killed anyway when harvested. As stock enhancement aims to replenish the stocks and create a sustainable fishery, the fishery can be used as a sampling method to assess the effectiveness of the stocking programme.

4.3.3.1. OTC in saline water

One of the drawbacks with OTC for the use in immersion of marine fish is that the OTC is only available for absorption by the fish when used in water with a salinity of less than 5 ‰. When the salinity is greater than 5 ‰, OTC binds to calcium and magnesium ions in the water (Taylor *et al.* 2005a), by a similar process to which it binds to the calcium in the otoliths of the fish. This makes the OTC unavailable to the fish and is therefore ineffective as a tag as it is not incorporated into the otolith. This however, does not pose a problem for juvenile *A. japonicus* as they show a wide salinity tolerance (Whitfield 1998) and even show preference for such low salinities during this early life stage (Griffiths 1997). Studies by Taylor *et al.* (2005a) have shown that when immersing juvenile *A. japonicus* in OTC, a salinity of 5 ‰ gives satisfactory results and can be used as an effective marking technique.

4.3.4. Visible Implant Fluorescent Elastomer (VIFE)

Simple and cost effective detection has made the use of externally visible tags particularly popular. Most externally visible tags are themselves externally situated on the fish, for example dart and anchor tags. These, however, have been found to be inappropriate for many species and for smaller fish, due to their large size, low retention rates, and because they have an adverse affect on the behaviour and survival of the fish (Doupe *et al.* 2003). Internal tags that are externally visible can eliminate some of these problems that are experienced with the external tags. A commonly used externally visible internal tag for tagging juvenile fish is that of visible implant fluorescent elastomer (VIFE) tags.

VIFE is a "medical grade, two-part silicone based material that is mixed immediately before use and then injected as a liquid that cures into a pliable, biocompatible solid" (Northwest Marine Technology, Inc. 2005; 1). The VIFE is injected, while in its liquid state, into transparent tissues on the fish such as the caudal fin, using a syringe with a 29 gauge needle, after which the VIFE hardens within a few hours. VIFE comes in 10 different colours including five fluorescent colours which make them visible under UV light even if they have been obscured by pigment in the growing fish (Northwest Marine Technology, Inc. 2004). The colours can be used in different combinations to individually mark cohorts or individuals. With improved technology tagging rates of up to 600 fish per hour can be achieved, although rates may vary by species, tag placement, fish size and tagger skill (Northwest Marine Technology, Inc. 2006).

VIFE has been effectively used on fish as small as 8 mm TL (Northwest Marine Technology, Inc. 2004). However, great care needs to be taken when tagging small fish, as they run a greater risk of being injured during the tagging process, than larger fish. The tag site should be selected according to the size and species of fish, taking into account pigmentation as the fish ages.

Table 4.3: Sites used for tagging with VIFE tags

Species	**Tag site**	**TL (mm)**	**Time (days)**	**Retention**	**Survival**	**Reference**
Micopterus salmonoides	Operculum	100	210	84.4%	77%	Catalano *et al.* 2001
Salmo trutta	Anal fin base	29-44	77	> 90%	99.5%	Olsen and Vollestad 2001
Acanthopagrus butcheri	Caudal fin	58	180	64%	100%	Doupe *et al.* 2003
Bluegill sunfish	Caudal peduncle	34-55	180	100%		Dewey and Zigler 1996
Colorado pikeminnow		50		87%		Haines and Modde 1996

VIFE tags have been successfully applied to many species of fish, for research, aquaculture and stock enhancement purposes, around the world (Table 4.3). In New Zealand, VIFE tags were used by Willis and Babcock (1998) in the assessment of the fishery for snapper, (*Pagrus auratus*). In a study by Catalano *et al.* (2001) it was found that tag retention in large mouth bass (*Micopterus salmoides*) and bluegills (*Lepomis macrochirus*) was generally limited to shorter periods, less than a year, as periods of longer than this showed unacceptably high tag loss. It was also found that *L. macrochirus* was more susceptible to predation by *M. salmoides* when tagged with brightly coloured tags as opposed to cryptically coloured ones. Further investigation should be considered for similar effects in *A. japonicus* before fish tagged with brightly coloured tags are released into the wild.

Table 4.4: Advantages and disadvantages of the three methods of tagging that were evaluated for juvenile *A. japonicus*.

Tagging Method	Advantages	Disadvantages
CWT	• Easy to detect • Can be identified on live fish	• Each fish must be tagged individually • Expensive setup costs (applicator and detector) • Poor tag retention and survival
VIFE	• Can be identified on live fish • Identified visually (no detector costs)	• Each fish must be tagged individually • Short-term tag retention • Fish need to be anesthetised before tagging
OTC	• Batch marking by submersion (no individual marking) • Inexpensive • Long-term tag retention	• Fish must be sacrificed in order for OTC to be detected • Otoliths need to examined under fluorescence microscope (requires time consuming preparation of otoliths)

The aim of this study was to identify tagging methods that can suitably be used for pilot studies prior to stock enhancement as well as for post-release monitoring of hatchery reared and released *A. japonicus* fingerlings in a stock enhancement scenario, as each method has its advantages and disadvantages for a particular purpose (Table 4.4) The study compares tag retention survival and growth among fish tagged with coded wire tags, visual implant fluorescent elastomers and OTC immersion.

4.4. Methods and Materials

Six hundred *A. japonicus* juveniles with a mean length of 97.5 mm (± 10.05 mm) SL were obtained from a commercial aquaculture facility, Espadon Marine of Hermanus, South Africa, and transported to the Rhodes University Marine Laboratory in Port Alfred. The fish were then divided into five 1000 L tanks which were a part of a semi-recirculation system on the banks of the Kowie River. Throughout the experiment the water temperature ranged between 13 °C and 16 °C. The tanks were kept covered with 80% shade cloth to reduce the light in the tanks as this is known to reduce stress in the fish, as well as to prevent fish from jumping out of the tanks. The fish were allowed 5 days to acclimate and recover from the stress associated with transportation. The fish were fed to satiation twice daily throughout the duration of the experiment with an artificial trout fry feed with a grain size of 1.8 mm (produced by Indian Ocean Feeds). The feed was comprised of 45% (min) crude protein, 14% (min) crude fat, 4% (max) crude fibre, 10% (max) ash and 10% (max) moisture.

One hundred fish were selected randomly for each of the four treatments of the experiment and their standard lengths (SL) measured to the nearest mm. Standard lengths were used to measure fish as many fish had damaged caudal fins as a result of cannibalism. There were no significant differences between the initial lengths of the experimental treatments (Kruskal-Wallis rank sum test: p-value = 0.4483) (Table 4.6 and Figure 4.1). Kruskal-Wallis rank sum test was used, as the assumptions of normality and homogeneity of variance were not met, therefore parametric tests (ANOVA) could not be used. Each treatment was kept in a separate 1000 L tank, all of which were part of the same mentioned semi-recirculating system. These 1000 L tanks were equipped with an air stone and covered with shade cloth to reduce stress and prevent fish from jumping out of the tanks (an observed characteristic of juvenile *A. japonicus*).

4.4.1. Coded Wire Tags

The fish in this treatment were not anaesthetised, as application of the coded wire tag was possible without the added risks and stress associated with anaesthetic. The fish were caught individually by hand and the coded wire tag was inserted into the cartilaginous area on the tip of the snout using a single shot manual tag injector (24 gauge needle). The snout is a common tagging site for many species; however, care should be taken as to the depth of insertion of the tag (Northwest Marine Technology, Inc. 2002). Inserting the tag too deep can cause injury to the fish, while not inserting the tag deep enough can lead to tag loss. The fish were handled with a wet, non-abrasive cloth to minimise the risk of secondary skin infections caused by damaging the mucus layer covering the fish, or removal of scales. The fish were then placed in a recovery bucket containing clean sea water from the recirculation system, and observed for any unusual behaviour, such as abnormal swimming, to ensure that they had not been injured or damaged during the tagging process, after which they were released into the 1000 L experimental tank. The fish were fed daily to satiation.

Five month after tagging, the fish in this treatment were caught with a scoop net and were sacrificed by placing them into ice water. The fish were then measured to the nearest mm (SL). Their heads were then removed with a scalpel, laid out on a tray, and X-rayed to locate and confirm the presence or absence of the tags. The metal tags were clearly identifiable in the X-rays, despite their small size, and were easily counted with the naked eye when the X-rays were held up to the light as shown in Figure 4.2.

4.4.2. VIFE Tags

One hundred *A. japonicus* juveniles were anaesthetised individually by placing them in a 5 L solution of 2-Phenoxyethanol (Cowley *et al*. 2006) at a concentration of 0.4 ml/L, prior to tagging. Fish were removed from the anaesthetic once they reached stage 4 anaesthesia (McFarland 1959); defined as "total loss of equilibrium – total loss of muscle tone and equilibrium; slow but regular opercular rate, loss of spinal reflexes" in Summerfelt and Smith (1990). Lower levels of anaesthesia would allow the fish to twitch during tagging, increasing the chance of injury, while higher levels of anaesthesia would increase the risk of cardiac arrest (Summerfelt and Smith 1990).

The most common tag insertion sites on smaller fish include the bases of the pectoral, pelvic and anal fins, the caudal fin rays, the underside of the jaw, and adjacent to the dorsal fin (Northwest Marine Technology 2004). However, due to loss of fins resulting from cannibalism, the fins were excluded as suitable sites for tagging. The jaw and the dorsal side of the fish were heavily pigmented creating unsuitable conditions for tagging. After testing several sites on different individuals not included in the experiment, the VIFE was injected subcutaneously in the ventral side of the fish, anterior to the anus (Figure 4.3). This surface is covered in smaller scales and is less pigmented; creating what seemed to be the most suitable site for insertion of the tag.

The VIFE was injected subcutaneously into the fish using a 29 gauge hypodermic needle. The needle was inserted anteriorly and the VIFE injected into the gap created by the needle as it was retracted, ending approximately 1 mm from the point of insertion, as the tag is likely to be shed if it is protruding from the hole (Northwest Marine Technology 2004). As recommended by Northwest Marine Technology (2005) the remainder of the VIFE, after all fish had been tagged, was kept and observed to ensure that drying took place within 24 hours of tagging. This ensured that the batch mixed for the experiment did in fact dry within the specified time and that tag loss could not be attributed to a faulty batch of VIFE.

Each tagged fish was put into a 5 L recovery bucket until it had recovered from the anaesthetic (reactive to external stimuli, opercular rate and muscle tone normal) (Summerfelt and Smith 1990), where after it was put into the 1000 L tank, where it would be kept for the duration of the experiment.

At the end of the experiment, fish were captured from the 1000 L experimental tank using a scoop net. They were then measured to the nearest mm (SL) and visually inspected for the presence of VIFE. The lack of pigment on the tag site allowed for easy visual identification of tags (Figure 4.3).

4.4.3. Oxytetracycline HCL

One hundred fingerling *A. japonicus* were introduced into an 80 L upwelling tank containing sea water (35 ‰ salinity) from the recirculating system. An air stone was used to aerate and maintain water circulation in the tank. Fresh water (0 ‰) was then siphoned into the upwelling tank slowly allowing the tank to overflow through a sieved outlet, bringing the salinity down from 35 ‰ to 5 ‰ over a 6 hour period. Salinity was monitored continuously during this period using a refractometer until the salinity was at 5 ‰. This time scale was chosen as it is similar to that of a tidal change in an estuary, similar to those favoured by juvenile *A. japonicus* and it is more conservative than the duration used in a similar treatment in an experiment by Taylor *et al.* (2005a), of 3 hours. Oxytetracycline HCL was then added in the form of 400 ml of Hi-Tet 120 (120 mg OTC/ml) at a concentration of 600 mg/L as suggested by Taylor *et al.* (2005a). The tank was covered to keep UV (sunlight) from degrading the OTC as well as to reduce stress in the fish (Kreiberg 2000) and to prevent the fish from jumping out of the tank. The fish were kept in the solution for a period of 24 hours, where after sea water was slowly pumped into the tank to bring the salinity back to 35 ‰ over a 6 hour period. The fish were then released into the 1000 L tank in which they were kept for the duration of the experiment.

After the duration of the experiment, these fish were measured to the nearest mm (SL) and sacrificed by putting them into ice water. Otoliths were then removed and embedded in polyester casting resin, sectioned with a double bladed diamond-edged otolith saw and mounted on glass microscope slides with DPX mountant. The otoliths were stored in the dark during the drying periods to minimise degradation of the OTC marker by sunlight. Slides were then examined under a fluorescence microscope with a UV light at 10x magnification to identify traces of OTC present in the otolith.

4.4.4. Control

As these fish were not tagged in any way, they were simply measured to the nearest mm (SL) and returned to their tank. At the end of the experiment these fish were counted and measured to record survival and growth for the duration of the experiment.

4.5. Results

4.5.1. Survival

The fish from each of the treatments were counted after the five month period. However, the facilities available did not allow for replication of treatments and there was only one tank for each of the treatments. Thus, a Chi-square goodness of fit test was applied to test for significant differences between the treatments and the control (X^2= 2.53, 0 and 0.91 for CWT, VIFE and OTC respectively; all which are < 3.841, from the X^2 table). It is, therefore, not possible to attribute mortality to the tagging method associated with the treatment. The survival rates for the treatments are presented in Table 4.5. Furthermore, the mortalities were apparent victims of cannibalism, as they showed signs such as partial or total absence of fins and small lesions on the body.

Table 4.5: Survival of juvenile *A. japonicus* for different treatments during the tagging study.

	CWT	**VIFE**	**OTC**	**Control**
Survival %	74	89	80	89

4.5.2. Growth

Each fish was measured to compare growth between the treatments, to detect any effect that the tags may have had on the growth of juvenile *A. japonicus* over the 5 month period, the results of which are shown in Table 4.6 and Figure 4.1. A Kruskal-Wallis rank sum test was used to test for differences among growth in the treatments as variances in the data were not equal. No significant difference was observed in the growth among the treatments or the control (p=0.4483), indicating that there was no treatment effect tags on the growth of the juvenile *A. japonicus* fingerlings over the five month duration of the experiment.

Table 4.6: Initial and final lengths of juvenile *A. japonicus* tagged with CWT, VIFE, OTC and a control of untagged fish over a 5 month period.

Tag	Initial Length (Avg. SL ± Std. dev.) (mm)	Final Length (Avg. SL ± Std. dev.) (mm)
CWT	98.25 ± 10.05	150.47 ± 14.51
VIFE	98.17 ± 8.63	151.27 ± 11.27
OTC	97.28 ± 10.63	154.15 ± 15.57
Control	96.38 ± 10.77	153.11 ± 14.10

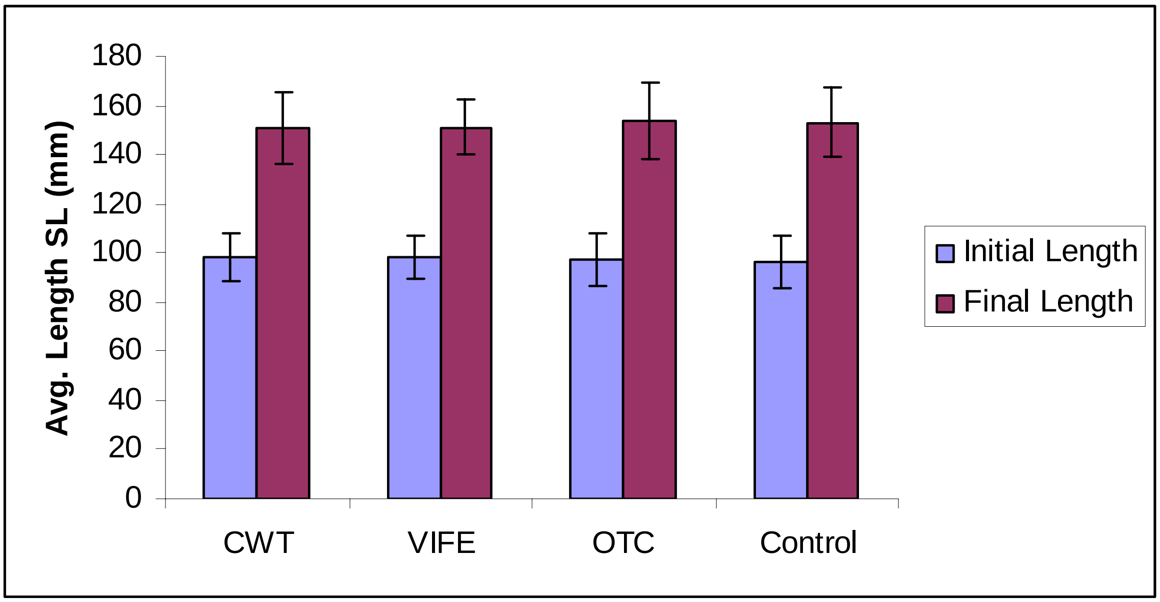

Figure 4.1: Initial and final lengths (mean ± S.D.) of juvenile *A. japonicus* tagged with CWT, VIFE, OTC and a control of untagged fish over a 5 month period.

4.5.3. Tag retention

Tag retention was measured for each of the treatments as described and is represented in Table 4.7.

Table 4.7: Tag retention observed for each treatment. VIFE (n=89), CWT (n=74), OTC (n=80).

	CWT	VIFE	OTC	Control
Retention %	60.81	61.80	100	—

Likelihood ratio tests showed that retention of tags was significantly higher for the OTC treatment than the VIFE tag treatment ($p<0.001$) and the CWT treatment ($p<0.001$); however, there was no significant difference between the VIFE treatment and the CWT treatment ($p=0.999$).

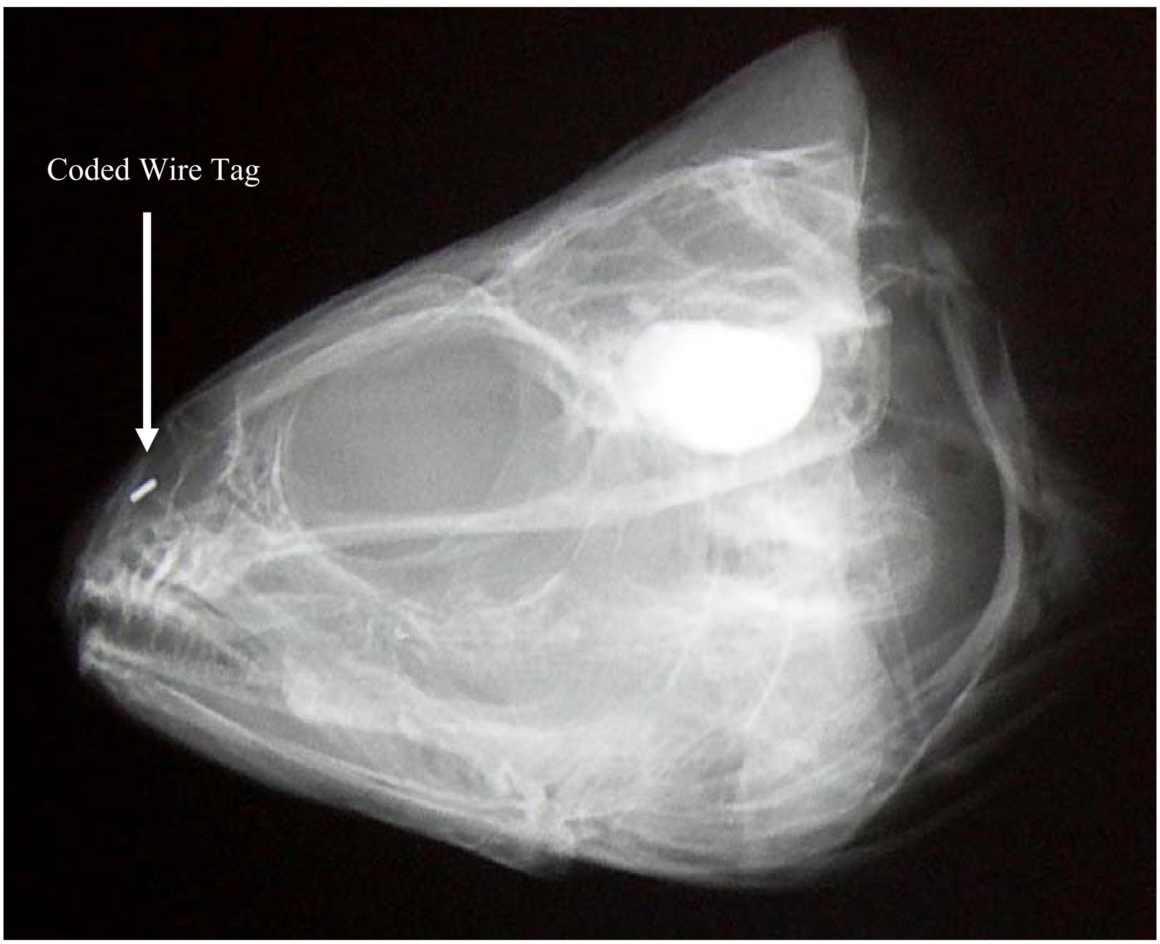

Figure 4.2: X-ray of juvenile *A. japonicus* head revealing coded wire tag in the snout.

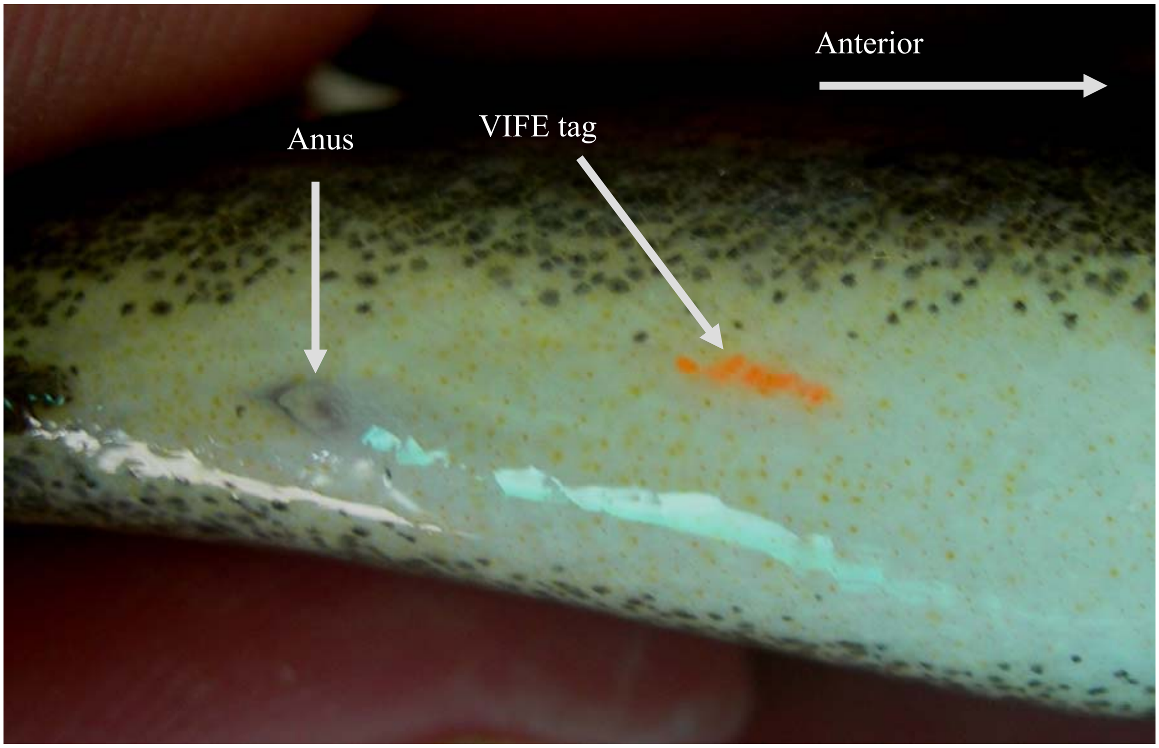

Figure 4.3: Ventral side of juvenile *A. japonicus* marked with orange VIFE tag.

4.6. Discussion

4.6.1. Survival

Survival for the treatments and control ranged from 74% to 89% and was considered acceptable given the relatively small size of the fish. No conclusions could be drawn on differences in mortality between treatments as there was a lack of replication in the experiment. The primary cause of fish death was attributed to cannibalism as juvenile kob are known to be highly aggressive and cannibalistic (Aquaculture SA, 2003), and this was supported by personal observations of their behaviour during the experiment. The majority of the mortalities showed some signs of being bitten (Figure 4.4).

Figure 4.4: Caudal fin of juvenile *A. japonicus* (154mm SL), showing signs of cannibalism.

4.6.2. Growth

Although the growth rate of the experimental fish was relatively slow due to the low ambient temperature during the experiment (range of 13 °C to16 °C), the experimental fish nonetheless increased significantly in length. The lack of any significant difference in growth rates among treatments (Figure 4.1) suggests that the tagging methods had no effects on the growth rates of the fish.

4.6.3. Tag retention

Fish tagged with OTC exhibited the highest tag retention (100% for OCT as opposed to 61.8% for VIFE and 60.81% for CWT). Although other studies CWT and VIFE have produced better retention results (Dewey and Zigler 1996, Haines and Modde 1996, Catalano *et al*. 2001, Olsen and Vollestad 2001), this study highlights the fact that these tags are poorly retained in juvenile *A. japonicus*. This could be attributed to the fact that they are physical materials in the body of the fish as opposed to a chemical stain as is the case with OTC and may be expelled as a "classic foreign body response". Regardless of the reason for tag loss in the VIFE and CWT treatments, whether it was due to poor tag application or simply being shed by the fish, these tags are unsuitable for post release monitoring, as tag application is a part of the tagging method and therefore the chance of tags being lost is equally high.

The importance of using effective tags for post release monitoring cannot be overemphasised. The loss of a high proportion of tags would result in the collection of inaccurate data and, since the management of the programme would be based on the information gathered during post release monitoring, consequently the mismanagement of the stocking programme. This could have severe ecological and environmental implications, as have been discussed in chapter 2.

4.7. Conclusion

Although VIFE and CWT may be capable of higher retention rates, this study has shown that there is potential for high tag loss. OTC on the other hand showed 100% tag retention after 5 months. As OTC was applied by immersion of fish in a solution, it would be equally easy to apply to a larger number of fish making it a highly suitable tagging method that could be used in a stock enhancement programme for *A. japonicus.*

In terms of survival, tag retention, cost and ease of batch application, OTC appears to be the most suitable tagging method for use in the stock enhancement of *A. japonicus*. This method can be applied to both pilot studies as well as for the post release monitoring of a full scale stocking programme.

CHAPTER 5

THE WILLINGNESS TO PAY FOR DUSKY KOB (*ARGYROSOMUS JAPONICUS*) RE-SEEDING: USING RECREATIONAL LINE FISHING LICENCE FEES TO FUND STOCK ENHANCEMENT IN SOUTH AFRICA

A manuscript based on this chapter co-authored by R.M. Palmer and J.D. Snowball has been submitted to the ICES Journal of Marine Science.

5.1. Introduction

Stock enhancement is a relatively expensive management tool, which if used in conjunction with traditional management measures, will increase the cost associated with fisheries management. Therefore, if the Department of Environmental Affairs and Tourism: Branch Marine and Coastal Management (MCM) is to financially support stock enhancement through the Marine Living Resources Fund (MLRF), an additional income into this fund will be required. The MLRF funds management of all sectors of the South African fishery, and users of the respective resources pay levies and licence fees which provide the income into the fund. Cross subsidisation between various sectors does occur as the poorer fisheries are funded by the richer ones. However, MCM is moving towards a "user pays" principle, and it is implicit that recreational fishing permits should be used to fund the management of the resources concerned.

Recreational anglers would be the primary beneficiaries of a kob stock enhancement programme as *A. japonicus* is a prime target species due to the life history strategy which restricts it to estuaries and surf zone until mature. But the question remains as to what the cost of the permit should be increased to. To make any stock enhancement programme economically feasible, the value would need to be such that it exceeds the cost of the programme, but because the existence of South Africa's ± 80 recreational line fish species is heavily dependant upon the traditional management measures in place, one cannot increase the permit fees to unaffordable levels, thereby excluding a large proportion of anglers from the fishery and reducing income from licences.

Since the permit system was implemented in 1998 at a fee of R30 for recreational angling without investigating the maximum amount that anglers would pay, it is possible that anglers would in fact pay a higher fee for the recreational angling permit. There is therefore a need for research into the maximum amount that recreational anglers would be willing to pay recreational angling permits, which could be used for improving linefish management including stock enhancement of *A. japonicus*.

Estimation of the economic feasibility of stock enhancement of *A. japonicus* is difficult as there is no data on the survival rate of hatchery reared fish and their contribution to the fishery. However, Cantrell *et al*. (2004) have shown that recreational anglers' demand for fishing quality may be used as a basis for the evaluation of the economic feasibility of stock enhancement. This has been done through the use of the willingness to pay valuation method. The aims of this research was therefore to evaluate the use of the willingness-to-pay method, to determine a suitable level to increase the recreational fishing permit fees to, and to determine how much extra a sample of fishers with valid recreational fishing permits would be willing to pay for the recreational angling permit in South Africa. This was done by surveying respondents' fishing habits, their opinions regarding the status of *A. japonicus* and the enhancement thereof. The data were used to determine whether a willingness to pay survey could produce reliable results that could be used to extend the scope of this pilot study.

To answer these questions, a pilot study of 102 face-to-face interviews, using a contingent valuation survey method, was conducted amongst permit holding shore anglers in the Plettenberg Bay and Nature's Valley area in the Western Cape during December 2005 and January 2006.

5.2. Overview of the willingness to pay method

The willingness to pay valuation technique is part of contingent valuation, which is the most widely used method of estimating the non-market values of environmental attributes or amenities (Frykblom 1997), for example, the value of an endangered species or a recreational resource. These values are usually based on user's willingness to pay for the improved service or resource, or their willingness to accept compensation for damage to the environment or resource (Frykblom 1997). This study was based on the resource users' willingness to pay for an enhanced *A. japonicus* stock, which could be a function of either the user's environmental concerns for the status of the stock, or their personal gain in terms of fishing success (enhancing both the recreational experience of fishing and the subsistence value of catching fish). This involves using data from a carefully designed questionnaire to estimate the amount that users may be willing to pay for a product or service that is not currently sold in the market (Fernandez *et al.* 2004). The method can be applied to public and private goods or to goods with mixed characteristics, as in this case.[2]

Contingent valuation is a survey or questionnaire-based approach to the valuation of non-market goods and services. The method involves giving randomly selected individuals information about a particular problem. They are then presented with a hypothetical occurrence, in this case the total collapse of the *A. japonicus* stock (where the stock no longer supports a fishery), and a policy action that ensures against the disaster, in this case stock enhancement. They are then asked the maximum that they would be willing to pay, in this case in the form of the recreational fishing permit, either to avoid the negative occurrence or bring about a positive one. Interestingly, the method can be used to measure use and non-use (also called option-use) values, so that, for example, people who do not use the resource frequently or at all, but still have a reason to want to preserve it, can indicate this through their willingness to pay.

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[2] Public goods are defined by Samuelson as "those goods that a number of people can use simultaneously without diminishing their value (non-rivalry) and once these goods are provided it is infeasible to exclude people from their use (non-exclusion)" (Duncombe 1996: 31).

Table 5.1: The pros and cons of the willingness-to-pay method as have been outlined in (Economic valuation of natural resources)

PROS	CONS
Based in economic utility theory and can produce reliable estimates.	Estimates of non-use values are difficult to validate externally.
Currently the only method available to measure important nonuse values associated with natural resources.	Stated intentions of willingness to pay may exceed true values. (Hypothetical bias)
Most biases can be eliminated by careful survey design and implementation.	Results may appear inconsistent with tenets of rational choice
Can be used to value a possible future scenario, not yet actually available.	Respondents may be unfamiliar with the good or service being valued and not have an adequate basis for articulating their true value
Is being constantly improved to make the methodology more reliable.	Respondents may express a value for the satisfaction ("warm glow") of giving rather than the value of the goods or service in question
Has been used successfully in a variety of situations.	Respondents may fail to take questions seriously because the financial implications of their responses are not binding.

Until 1990, WTP valuation methods formed a branch of environmental economics that was not studied in tremendous detail. However, after the *Exxon Valdez* ran aground in Prince William Sound, Alaska, in 1989 releasing 11 million gallons of crude oil, and the WTP method was used in litigation to quantify the damage caused to the environment, there was extensive debate as to whether contingent valuation surveys provide valid economic measures of people’s values for environmental resources that they may never actively use (Carson *et al.* 1996). In 1992 a panel of social scientists, including two Nobel laureates was appointed to critically evaluate the validity of contingent valuation

(Carson *et al.* 1996). This panel indicated several situations and biases that would provide unreliable results in a WTP survey. Because biases were identified, they should be investigated and means to minimise their effects identified.

5.2.1. Bias in the contingent valuation method

While biases have been observed in both directions, respondents usually overstate their willingness-to-pay in hypothetical examples. This can be attributed several hypotheses. The first of these is the "warm glow" hypothesis, whereby one finds that WTP responses reflect the willingness to pay for the moral satisfaction of contributing to a good cause, rather than the economic value of the good or service in question (Kahneman and Knetch 1992). Secondly, if the chance of the resource being improved is increased if the willingness-to-pay responses are higher, then respondents may state higher willingness-to-pay values (Murphy *et al*. 2004). Thirdly the "embedding effect" is when respondents' WTP responses are similar for surveys that are valuing different sizes of goods, in which theory suggests that responses should be different (Kahneman and Knetch 1992; Bateman *et al.* 2005; Snowball 2005).

Extensive research, using experimental techniques has shown that although biases do exist in the contingent valuation method (Bohm 1972, 1979; Thompson *et al.*, 1983; Throsby and Withers, 1986; List and Shogren 1998; Snowball, 2000; Botelho and Pinto 2002), they can be minimised through careful questionnaire design and implementation (Carson *et al*., 2001) as well as through the use of reliability and validity testing (Cummings and Taylor 1999; Aadland and Caplan, 2003). List and Gallet (2001) as well as Murphy *et al*. (2005) have shown that with carefully designed questionnaires, bias is usually small, with WTP values usually between 1.26 and 1.35 times that of real market situations.

Bias was minimised in this survey of recreational anglers by employing various recommended methods in the questionnaire design, including:

- The use of a familiar payment vehicle (recreational fishing permit).
- Only including holders of valid recreational fishing permits in the study.

- Using a face-to-face interview approach.
- The use of qualitative response questions (Box 3 and 4)
- Respondents instructed to respond as if they were actually spending the money (Brown *et al*. 2003).
- Use of a post decision confidence measure (Box 2)
- Including questions that can be used for validity and reliability testing.

5.3. Questionnaire design and sampling

There are several steps that should be followed when developing a survey of this nature in order to collect data that is as accurate and reliable as possible. Wedgewood and Sansom (2003) recommend the following steps that were taken into consideration in the development of the present survey.

Preparation:

1. Select the interview technique (phone, post, on-site interview, etc.)
2. Develop sampling strategy (sample size, random selection, etc.)
3. Develop a scenario (to explain how stock enhancement will help)
4. Define service options (how many extra fish will they catch in the future)
5. Cost the options (calculate realistic prices)
6. Develop questionnaire

Implementation:

7. Pilot testing and enumerator training (consider translation, etc.)
8. Conduct survey

Selecting the interview technique is an important decision as it can affect the response success from the participants. The options available to us are: face-to face interviews, telephonic interviews or a postal questionnaire. Face-to-face interview are generally the most effective method (Arrow *et al.* 1993) as it is easier to explain the background information that is required. Respondents of face-to-face interviews are more likely to respond to the questionnaire as well as to answer questions truthfully, however avidity bias can come into play with this type of sampling. Although telephonic interviews have some advantages in terms of costs and centralises supervision and elimination of avidity bias, it is likely that in South Africa, a large proportion of recreational anglers do not have access to a telephone due to their poor socio-economic status and thus telephonic interviews would skew results to more affluent participants. Postal surveys are expensive and tend to have a low response rate especially if the questionnaire is long, and coupled with this it is difficult to convey background information to respondents in a postal survey. It is agreed that it is unlikely that reliable estimates can be elicited with mail surveys (Arrow *et al.* 1993). Postal surveys can also bias against illiterate anglers, who in South Africa may represent a significant proportion of recreational anglers. In a survey done on willingness to pay for fishing on reservoirs on the Snake River in the U.S.A (US Army Corps of Engineers 1997), a postal interview was successfully conducted. This was thought to be due to initial on-site contact with the fishermen, a US$ 2 incentive included in the envelope, and a university return address (US Army Corps of Engineers 1997). However in this study, no prior contact had been made with fishermen, and no incentives could be offered to fishermen for answering the questionnaires, therefore a postal survey was not likely to have been successful.

Face-to-face interviews were therefore conducted with 102 licensed recreational shore anglers in the Plettenberg Bay and Nature's Valley areas in the Western Cape, South Africa during December 2005 and January 2006. The holiday period was chosen to conduct the survey as at this time a representative sample of both holiday makers and frequent fishers were be present. All anglers (regardless of race or gender) on the shore, on interview days, where approached whether they were fishing in the estuary, in the surf, or from the rocks. Only anglers with in-date fishing permits were interviewed on the grounds that those not willing to pay for the existing licence would probably not pay an extra amount for stock enhancement. A total of 160 anglers were approached during the survey, however, of these only 102 had valid angling permits. Only anglers who were over the age of 18 years were included in the study, as younger anglers were less likely to have paid for their own recreational fishing permits. On approaching the anglers, it was made clear that the purpose of the study was purely to gather information and not for compliance purposes, the survey was anonymous and no action could or would be taken against any anglers who reported to being non-compliant, and respondents were encouraged to answer the questions truthfully.

The questionnaire (Appendix 1) consisted of six sections, namely; A) an introduction, background information, the purpose of the study and a request for participation in the interview; B) qualitative response opinion questions (agree, disagree, don't know); C) specific questions on kob fishing habits; D) the WTP scenario and questions; E) qualitative reasons for being willing or unwilling to pay and F) demographic information.

The WTP scenario explained the status of the kob stock in South African waters and the need for an alternate management plan. Stock enhancement was briefly explained as the contingent market scenario. The respondents were then made aware of the potential benefits to them as anglers, should the programme be implemented. In order to minimise the effects of information bias as much as possible, that is, influencing responses by the way in which information is provided (Kenyon and Edwards-Jones 1998), the programme was simply and briefly outlined as shown in Box 5.1. As mentioned, in order to make the scenario as plausible as possible, as well as to avoid the hypothetical bias, a familiar payment vehicle which is necessary for all recreational anglers to pay in the form of an increase in the price of fishing permits, was used.

Arrow *et al*. (1993) report that when stating their WTP, in many cases respondents may neglect the budget constraints that they may have. This is often detected, as the WTP amount may make an unreasonably large proportion of the respondent's income. A suggested method of minimising this bias is to remind the respondents of budget constraints. This was done in this particular study as can be seen in Appendix 1, where respondents where asked to consider their annual income and expenses when stating their maximum WTP for stock enhancement. Validity and reliability tests using the data form the demographics questions were also used to confirm that this was in fact effective.

Box 5.1: The contingent valuation scenario from the survey.

CONTINGENT VALUATION SCENARIO FROM THE KOB SURVEY

At present the spawner biomass per recruit of kob stocks has been reduced to less than 4% of pristine, indicating severe stock depletion, and that current management is not facilitating their recovery.

Stock enhancement is a management method whereby kob will be bred in captivity in a hatchery until a certain size and then released into the wild to boost the stocks. This will result in an increased population and eventually the full recovery of the species so that it may be harvested sustainably.

The benefit to you as an angler would be that kob catches would become more frequent. Although the daily bag limit will not be increased, it may be attained more often.

But stock enhancement is an expensive management tool, so fishing licences would have to be increased.

As shown in Box 5.2, the first WTP question used a binary yes/no response format, as this has been shown to produce more reliable results because it mimics the kind of market decisions that consumers are familiar with making (Arrow 1993). This was followed by an open-ended question in order to increase the accuracy of the model. Once a decision had been made by the respondent as to the maximum amount that they would be willing to pay for the fishing permit, a "post decision confidence measure" (Bennett and Tranter 1998: 255) was used to identify respondents who were unsure of their answers; a method which has been shown to be an effective technique of decreasing hypothetical bias (Champ and Bishop 2001). Only the results obtained from those respondents who were "fairly sure" or "very sure" of their WTP responses were included in the study.

Box 5.2: The Willingness-to-pay question from the survey

THE WTP QUESTION FROM THE KOB SURVEY

Now I am going to suggest an amount by which licence fees would increase in order to pay for stock enhancement. The amount may sound much too low or much too high to you. It is just a starting point and you can tell me what the maximum amount you would be willing to pay would be.

Now, considering your annual income and expenses, would you be willing to pay and extra R 40 per year to support stock enhancement? This means that the yearly licence fee would increase to R 100.

□ yes □ no

1. What is the maximum additional amount you would be willing to pay in increased licence per year?
2. How sure are you that you would really be willing to pay the amount that you mentioned? (read the options)

□ not at all sure □ fairly sure □ very sure □ don't know

Finally, important debriefing questions were asked, probing reasons for being willing or unwilling to pay. This is important, not only because it adds to the explanatory power of the survey, but also because it can be used to detect biased responses (Arrow 1993), for example, respondents who did not accept the contingent market ("I do not think that the money will be spent on the project"), respondents who used the survey to express protest of various sorts (for example against the idea of fishing licences) and possible "warm glow" responses, i.e. where respondents indicate that they "like to support a good cause", rather than valuing the programme in hand (Chambers and Chambers 1998).

Section F collected demographic information, such as age, race, and sex, level of education, job, monthly household income and household size, shown in Appendix 1, which was used in the analysis of the WTP responses.

A potential source of bias that arises in on-site surveys is that of avidity bias, when avid anglers are overrepresented in on-site surveys (Thompson 1991), as anglers are not sampled randomly, but rather in proportion to their frequency of fishing (Pollock *et al*. 1994). This can lead to a possible over estimation of willingness-to-pay, as avid anglers are likely to be prepared to pay more.

To avoid avidity bias in this the analysis of the results of this survey, the willingness-to-pay results for frequent and non-frequent anglers were kept separate. The two groups were then analysed separately so that the overrepresentation of frequent fishers did not bias the overall willingness-to-pay results

One of the flaws in the use of contingent valuation for estimating WTP for stock enhancement, and particularly of a species where there is to date very little information on the potential success stock enhancement of the species, is that the valuation has to be done under the assumption that stocking will be successful. The WTP results would likely be lower if it was indicated to the respondent that there was a certain probability that stock enhancement would be unsuccessful. This reinforces the need for studies into the potential successfulness of stock enhancement of *A. japonicus*, so that respondents can be informed of the probability of a successful stocking program when indicating their willingness-to-pay.

5.4. Results

5.4.1. Socio-Economics of Survey Respondents

The majority of the anglers that were interviewed were either of European-origin (61.8%) or mixed-origin (34.3%) with African-origin individuals making up only a small proportion (3.9%) (Figure 5.1). Demographically (education and job), the mixed-origin group and the African-origin groups were similar to one another (both $X^2 < 3.841$), and for the purpose of this study they were pooled into a single group (non-white). This left us with a white (European-origin) and a non-white (African-origin and mixed-origin) group. The survey data that were collected showed two clearly distinguishable groups: those that fished less than 25 times a year (non-frequent fishers) and those that fished 25 or more times in a year (frequent fishers). A breakdown of sample demographics of the two groups is presented in Table 5.2 and in Figures 5.2, 5.3 and 5.4. It was observed that the frequent fisher group had a more even age distribution, while the non-frequent fisher group was dominated by anglers between the ages of 36 and 50 years (Figure 5.2).

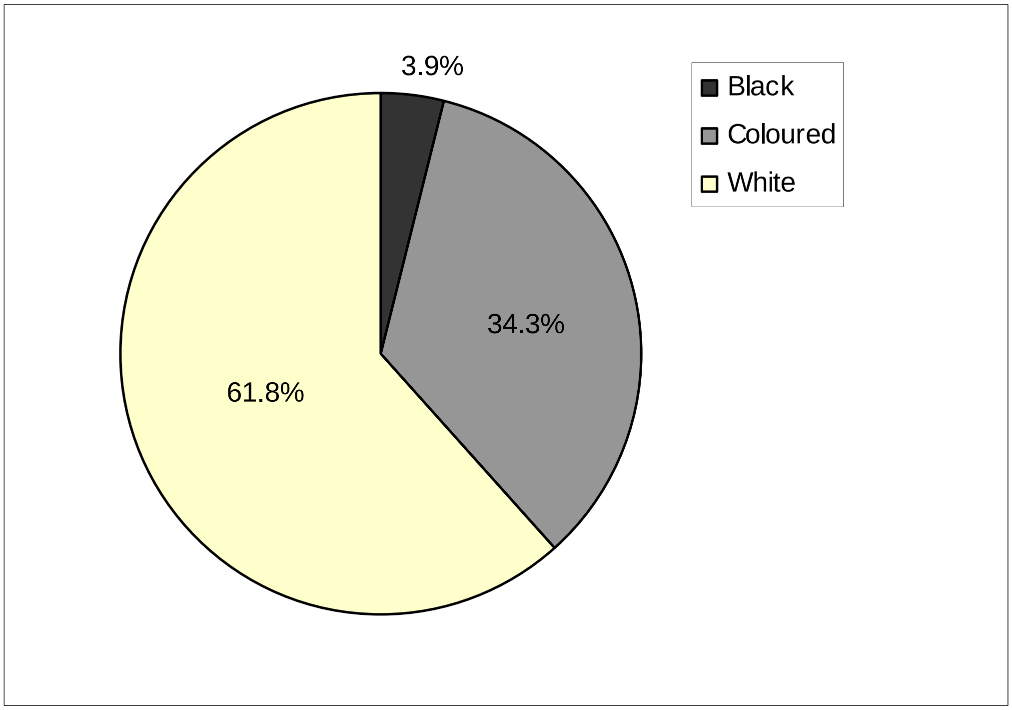

Figure 5.1: Race structure of Willingness-to-pay respondents

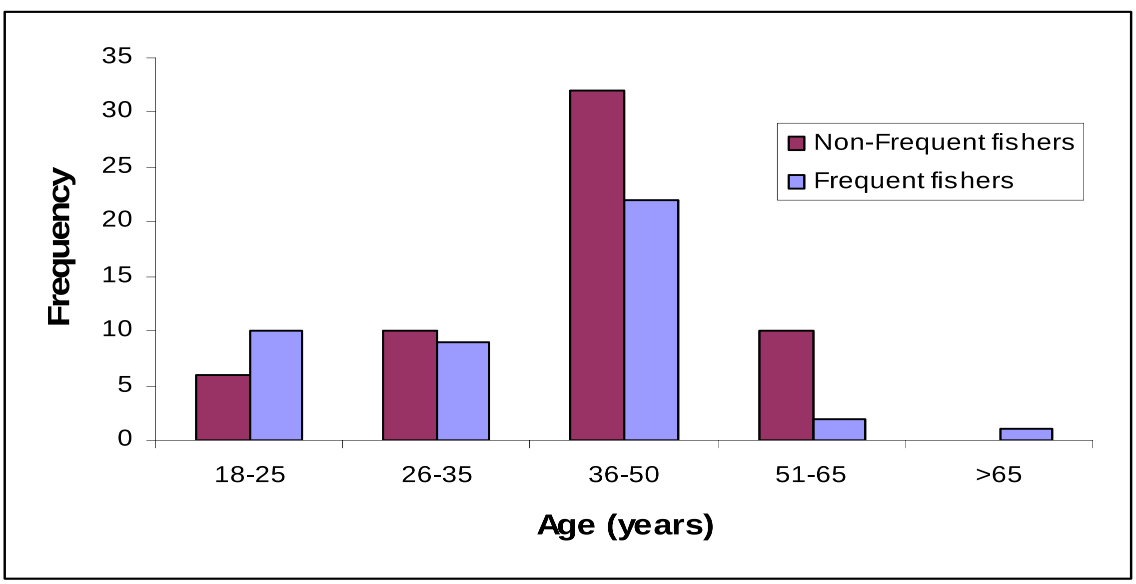

Figure 5.2: Age structure of frequent and non-frequent fishers

As a large proportion of the respondents did not disclose their incomes, their job description and level of education were used as a proxy for income as this was disclosed by all of the respondents. Job description was divided into a high and a low income category, with management, professional and self employed falling into the high income group, and employee, technical/trade, labour, unemployed falling into the low income group. The jobs were categorised using the data obtained from the proportion of respondents who were willing to disclose their incomes. This data confirmed that the jobs as defined fell into the designated higher and lower income groups. Similarly the level of education of the respondents helped to separate respondents who earned high and low incomes. Those who had obtained a higher level than a high school diploma earned a higher income, while those who had not completed a matric earned a lower income.

The frequent fisher group were more dependent on the fish that they were catching as a food source (subsistence), as the majority tended to fall into the low income job categories (employee, technical/trade, labour, unemployed) (Figure 5.4). Approximately 40% of fishers in this group had a higher level of education than a high school diploma (Figure 5.3). 58% of respondents were of European-origin, the remainder being mostly mixed-origin with a small minority of African-origin people. This is significant because (as is still generally the case in South Africa) European-origin respondents in this sample tended to have higher levels of income and education than other race groups. The majority of frequent fishers (68%) were local to the Plettenberg Bay and Natures Valley area, while only 43% of the non-frequent fishers were from the Plettenberg Bay and Natures Valley area.

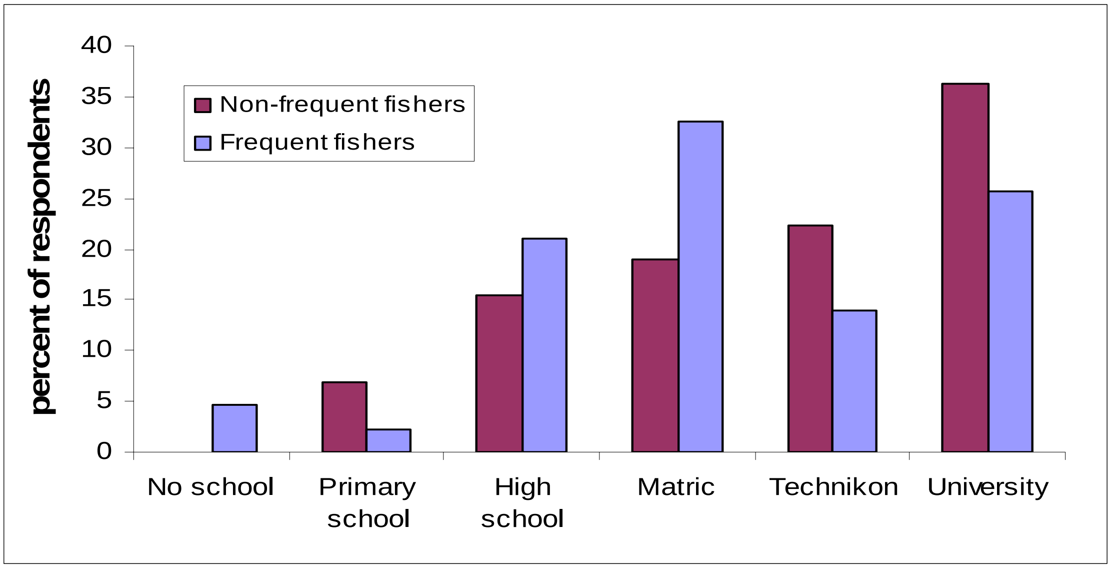

Figure 5.3: Percentage of highest level of education obtained by frequent and non-frequent fishers

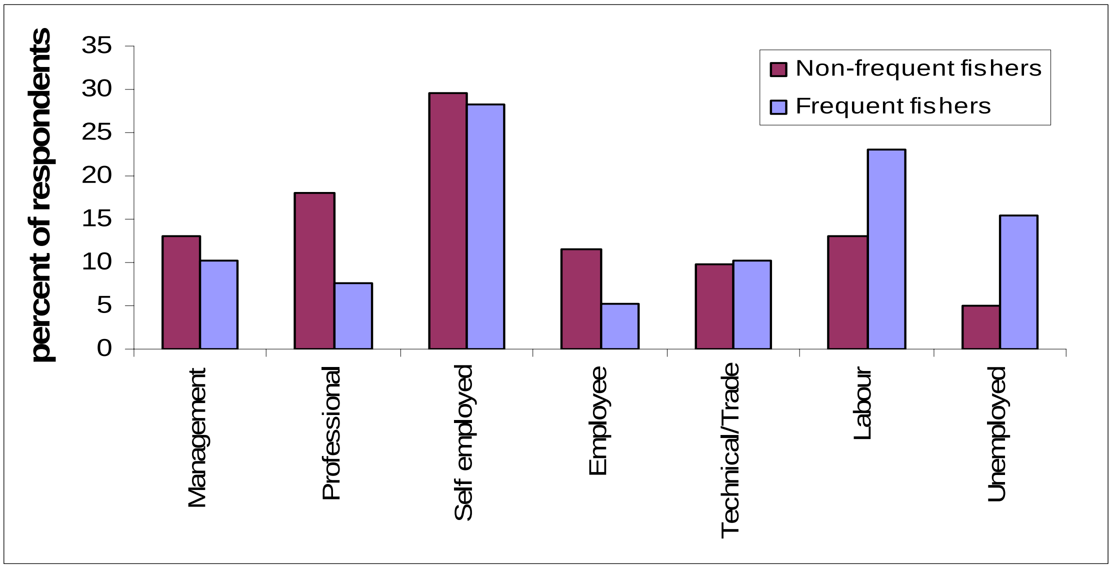

Figure 5.4: Percentage of frequent and non-frequent fishers in designated job descriptions

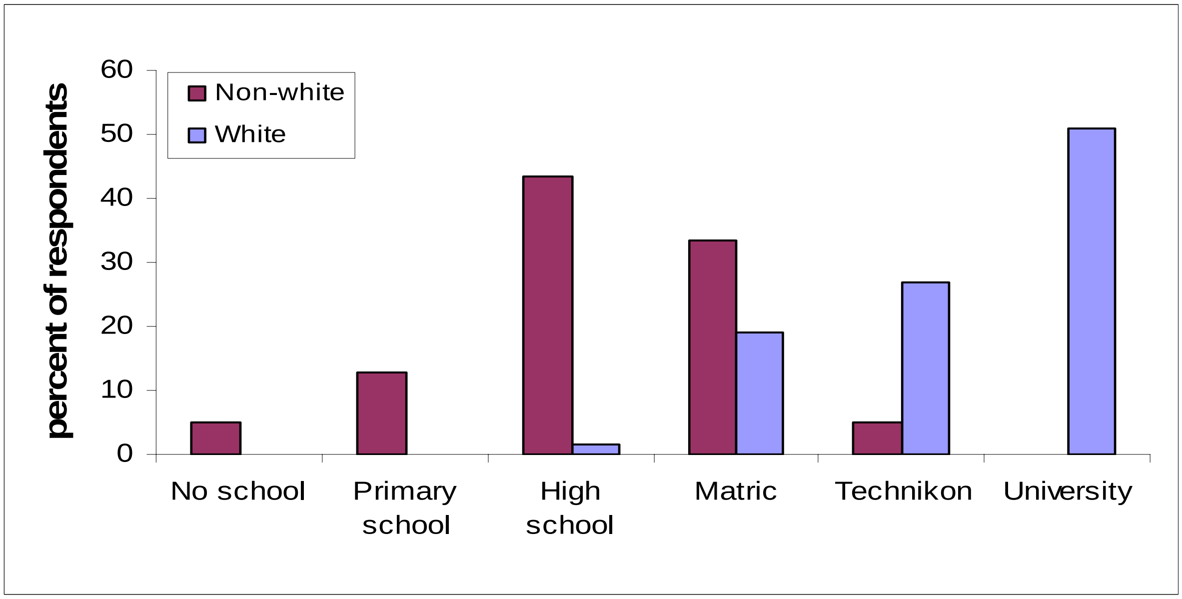

Figure 5.5: Percentage of highest level of education obtained by white and non-white respondents

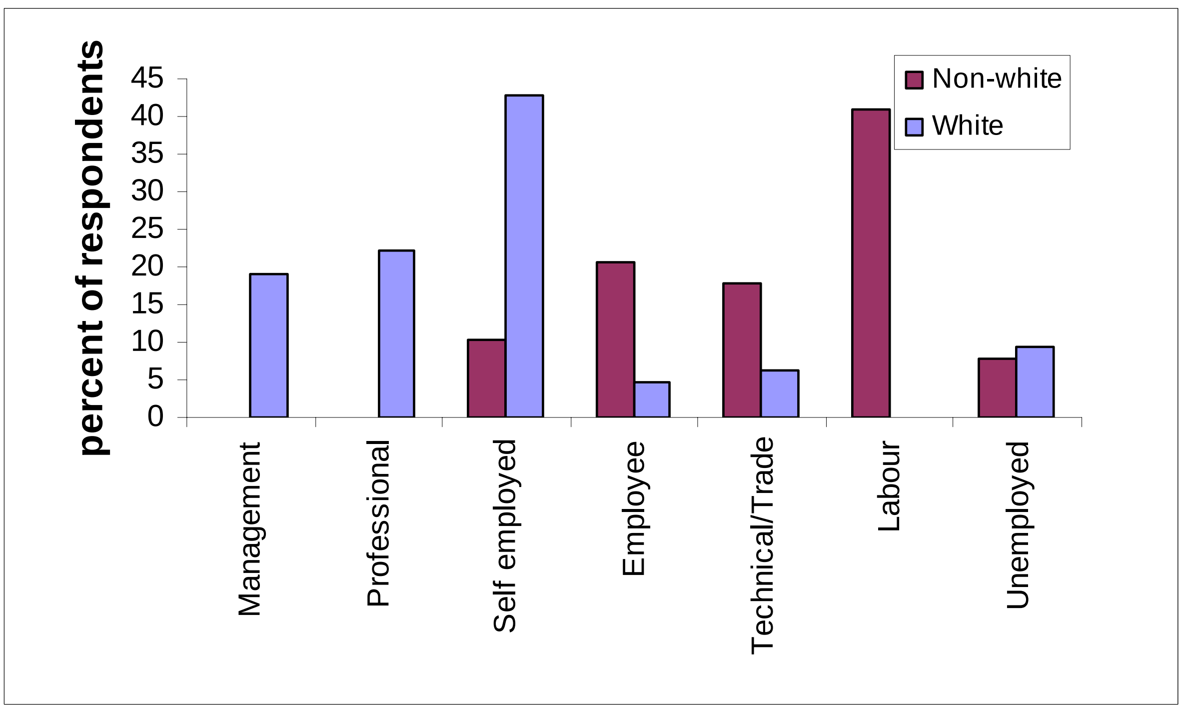

Figure 5.6: Percentage of white and non-white respondents in designated job descriptions

Most non-frequent fishers were fishing mainly for recreational purposes and a significantly higher number of them (63%) were holiday makers from outside of the region ($p = 0.0266$). Almost all respondents (95%) in this group were employed, with 58% being employed in high income jobs (self-employed, professional and management categories). There was also a higher level of education than amongst frequent fishers, with 58% of non-frequent fishers having some form of tertiary education.

Table 5.2: Socio-economic profile of survey respondents

	All (n=102)	Frequent (n=42)	Non-frequent (n=60)
Education > high school	50%	39.5%	57.6%
Employed	91.2%	88%	95%
High income job[3]	53.9%	46.5%	57.6%
Race: European-origin	61.8%	58.1%	64.4%

5.4.1.1. Responses to qualitative opinion questions and fishing habits

Responses to qualitative opinion questions revealed significant differences between the frequent and non-frequent fisher groups (Table 5.2). About three-quarters (76%) of all anglers in the sample reported that they complied with regulations, a slightly higher percentage amongst frequent (78%) than non-frequent fishers (73%). A significantly greater proportion of frequent fishers (91%) also agreed that fishing regulations were needed and indicated concern over the status of kob stocks than non-frequent fishers (72%) (Figure 5.7). This was possibly because respondents in the latter group were ignorant of regulations as well as the status of *A. japonicus* stocks and did not depend heavily on the fish for subsistence, as did a large proportion of the frequent fishers. The vast majority of all anglers interviewed (89%) reported that they never or hardly ever reached their bag limit (Figure 5.12), further illustrating the need for an alternate management strategy for *A. japonicus*.

[3] High income jobs: Self employed, professional and management.
Low income jobs: Employee, technical/trade, labour and unemployed.

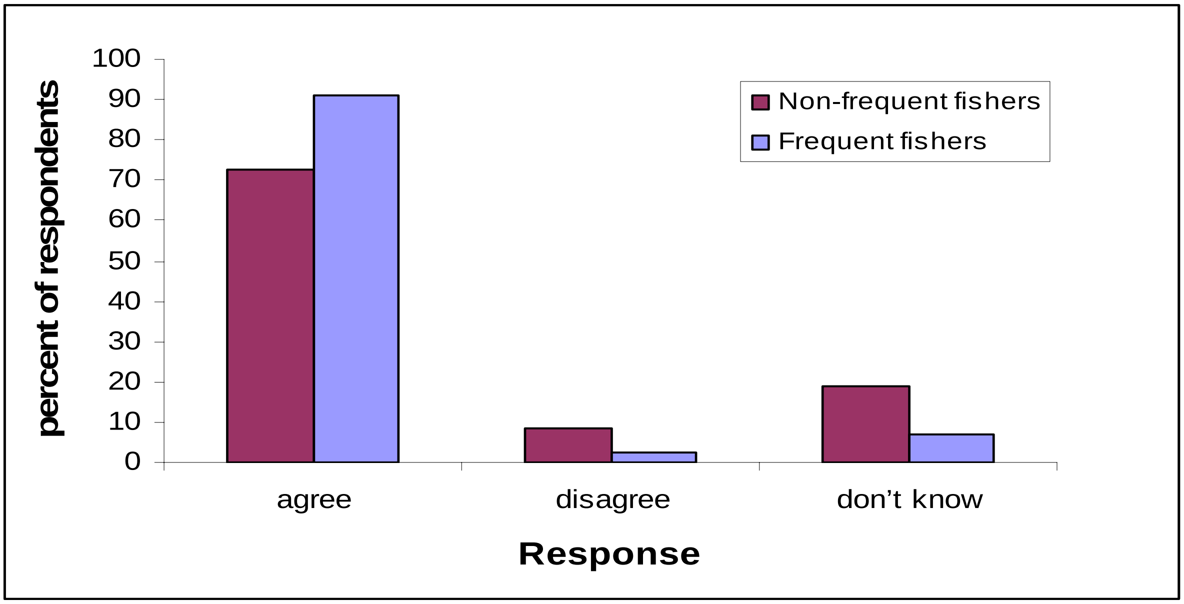

Figure 5.7: Responses of frequent and non-frequent fishers when asked whether they agreed that the current fishing regulations are necessary.

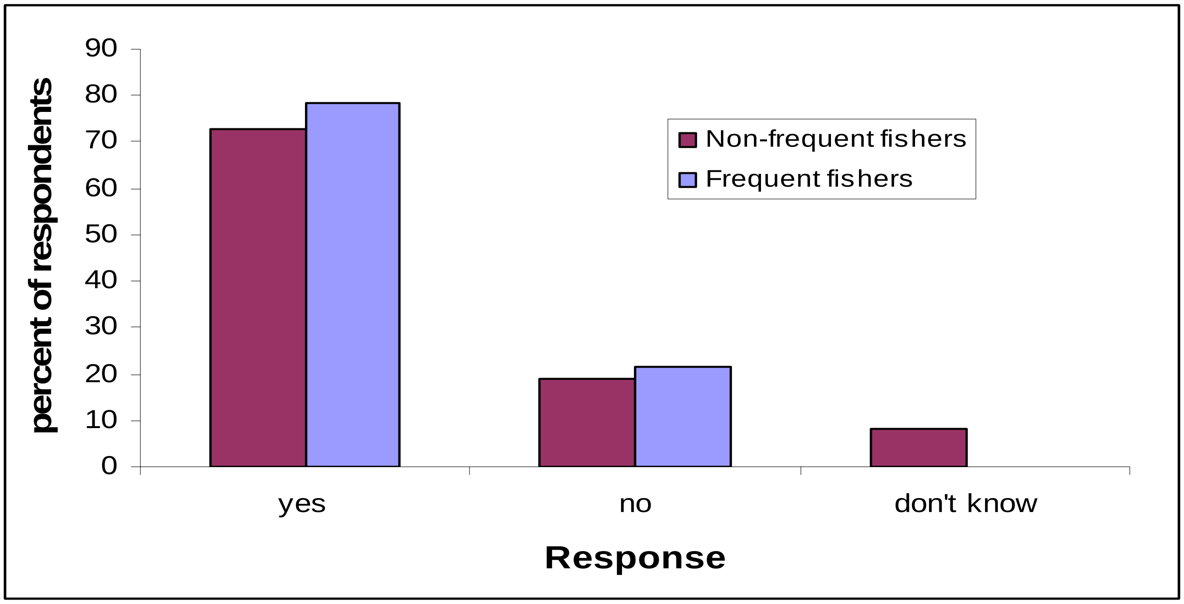

Figure 5.8: Responses of frequent and non-frequent fishers when asked whether they complied with the current fishing regulations.

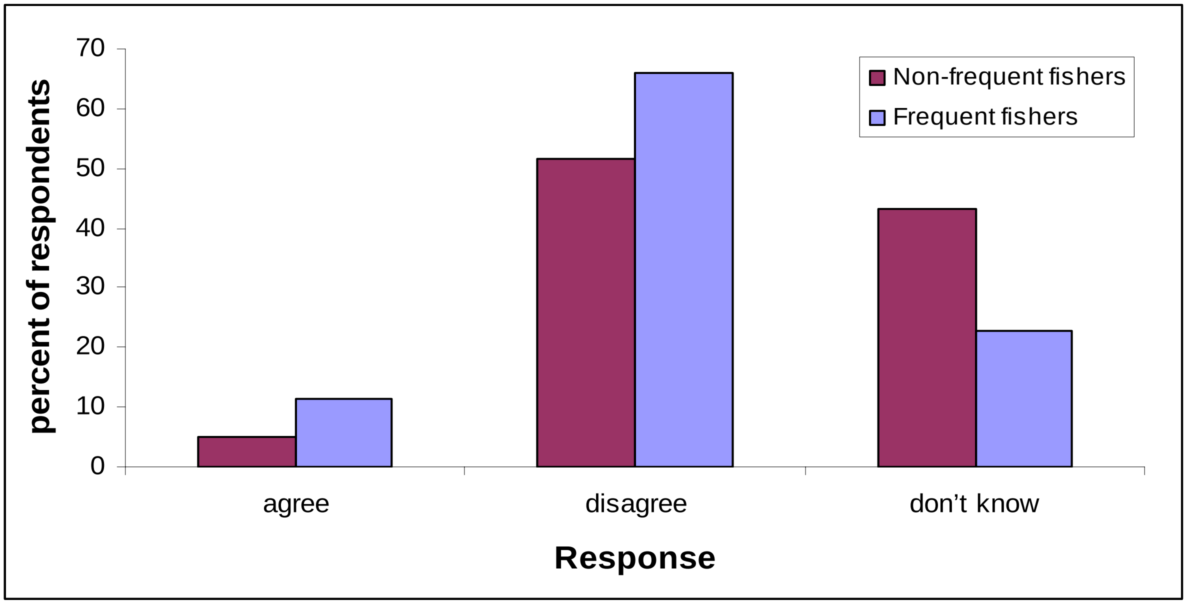

Figure 5.9: Responses of frequent and non-frequent fishers when asked whether they agree that the current status of kob stocks is healthy.

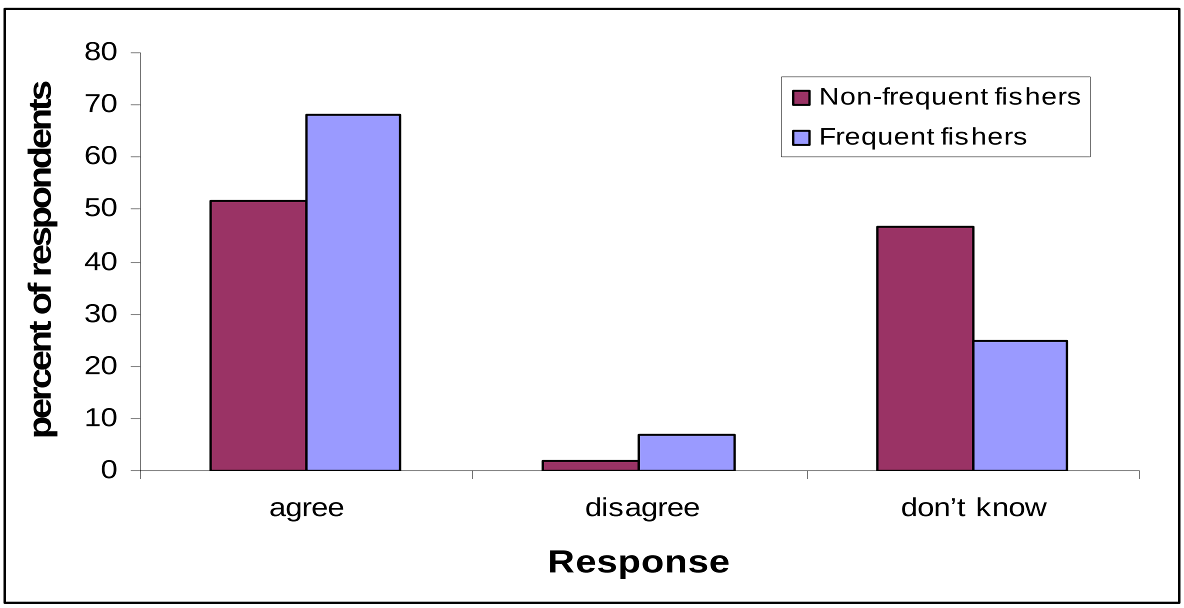

Figure 5.10: Responses of frequent and non-frequent fishers when asked whether they agree that something should be done to facilitate the recovery of the kob stocks.

Table 5.2: Results of opinion questions and kob targeting for all respondents, frequent and non-frequent fishers

Question	All	Frequent	Non-frequent
Do you comply with regulations? (% yes)	76	78	73
Do you agree that regulations are needed? (% yes)	80	91	72
Do you think that kob stocks are fine as they are? (% no)	58	66	52
Do you agree that something needs to be done about kob stocks? (% agree)	59	68	52
Do you target kob? (% yes)	50	55	47
Do you reach your bag limit? (% in each category)	Never/hardly ever: 89.1 Sometimes: 5.9 Often: 5	Never/hardly ever: 90.7 Sometimes: 7 Often: 2.3	Never/hardly ever: 87.9 Sometimes: 5.2 Often: 6.9

The largest proportion (47%) of anglers fished from the rocks, while 30% fished in the surf, 18% in estuaries and 5% fished deep sea from a boat. Frequent fishers went on an average of 93 fishing trips per year spending a total of R 2 913, while non-frequent fishers went on 8 trips per year and spent R 1 203. The amount spent per trip was significantly lower in the frequent fisher group (R 63) than the non-frequent fisher group (R 333).

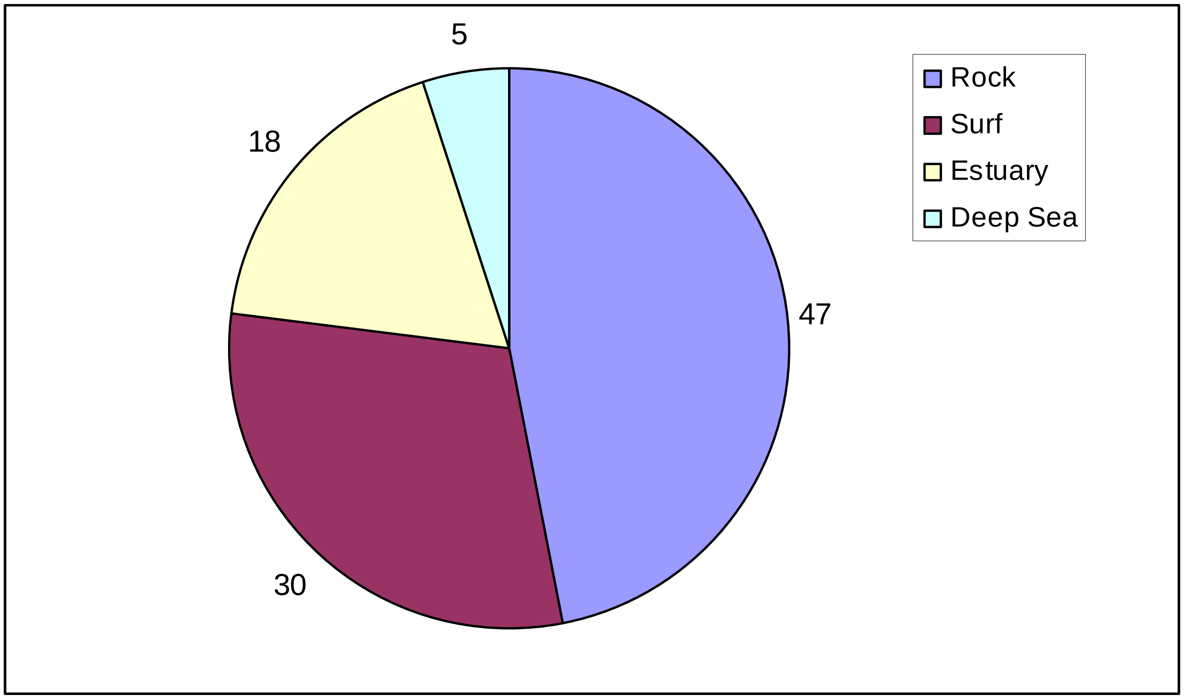

Figure 5.11: The percentage of respondents that fish mainly off the rocks, in the surf, in the estuary and deep sea.

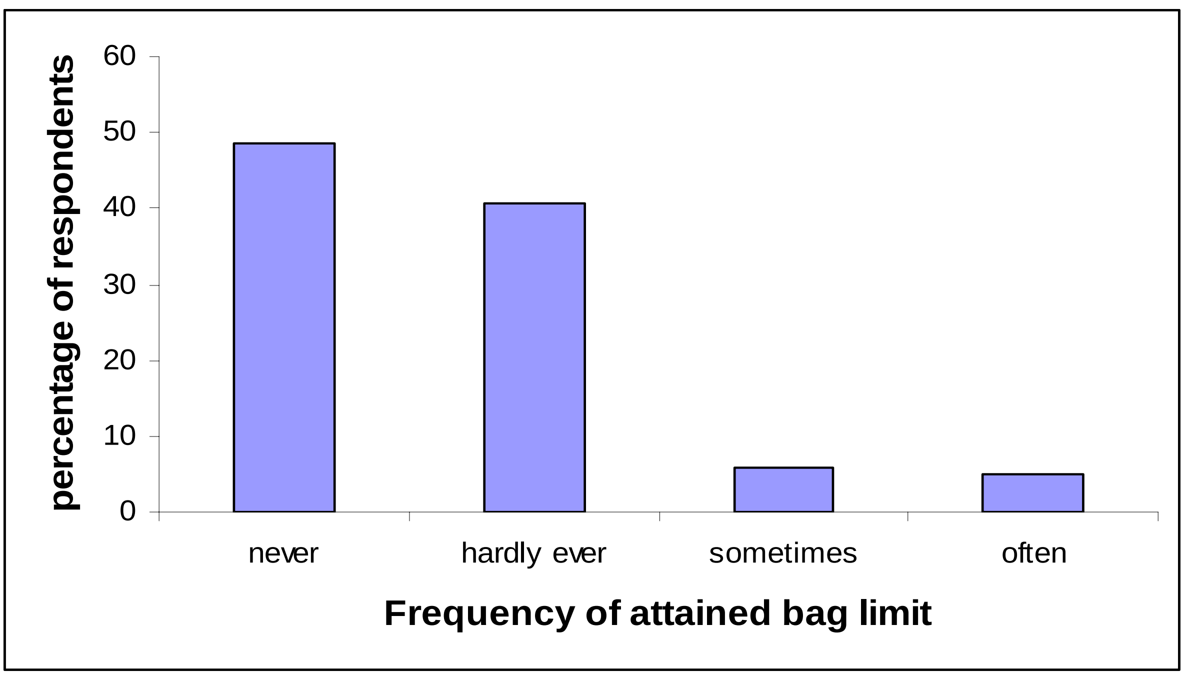

Figure 5.12: Percentage of frequency that daily bag limit is reached by the respondents

5.4.2. Willingness to pay results

The survey results showed that 75% of all the respondents were willing to pay more than the current R 60 for the recreational fishing permit. Mean additional willingness to pay for increased permits was R 162 for the whole group, R 183 for frequent and R 146 for non-frequent fishers. Median amounts (generally considered more reliable since they are not influenced by outliers) were somewhat lower (Table 5.3). In both cases, results showed that respondents who use the resource more are willing to pay more (Table 5.3), as economic theory would predict.

Table 5.3: Mean and median WTP results for all respondents, frequent fishers and non-frequent fishers

	All	**Frequent**	**Non-Frequent**
Mean WTP in ZAR	162. 21	182. 84	146. 55
Median WTP in ZAR	107. 50	155. 00	100. 00
Standard Deviation WTP in ZAR	126. 38	133. 06	119. 89

In examining the determinants of willingness-to-pay more for the angling permit, several model specifications were assessed, including linear ordinary least squares and binary response logit models, but the best goodness-of-fit with the most significant variables was obtained with a log-linear model; the results of which are reported in Table 5.4. Only those variables which were significant, or not significant but improved goodness-of-fit, were included in the final version of each model.

A log-linear model was used to estimate the willingness-to-pay as a function of the number of fishing trips per year ("trips pa"); whether respondents agreed that recreational fishing regulations were important ("regulations", where agree = 1, 0 otherwise); whether the respondent was aware that kob stocks were being depleted ("stocks", where yes = 1, 0 otherwise); whether the respondent agreed that something should be done to facilitate recovery of kob stocks ("recovery", where yes = 1, 0 otherwise); whether the respondents reported complying with recreational fishing regulations ("comply", where yes = 1, 0 otherwise) and finally some demographic information, including job (1= high income, 0 = low income); race (European-origin = 1, 0 otherwise); sex (male = 1; 0 otherwise); education (tertiary education = 1; 0 otherwise) and age group (where 26-65 years old = 1; 0 otherwise). In this case dummy variables were used for the non-continuous or categorical factors. These determinants were similar to some of those used in a similar study by Cantrell *et al.* (2004).

Table 5.4: Results of the log-linear WTP models

Variable	All (n=102)	Frequent (n=42)	Non-frequent (n=60)
Constant	4.03***	3.85***	3.13***
Trips pa	0.001**		
Regulations	0.34***	0.24	0.41***
Stocks	0.16	0.58*	0.15
Recovery		-0.58*	-0.025
Comply			-0.22
Job	0.26**		
Race	0.43***	0.40**	0.72**
Sex		0.32**	0.82***
Education		0.60***	-0.17
Age		0.20	0.23
R-squared	0.33	0.43	0.23
F-stat	10.76***	5.47***	3.22***

*** Significant at the 1% level

** Significant at the 5% level

* Significant at the 10% level

As the results in Table 5.4 show, all three models (all, frequent, and non-frequent) were statistically significant as all F-statistics were significant at the 1% level. Although they mat appear low, for cross sectional data with small sample sizes, and especially regarding peoples opinions such as this, the model fitted the data fairly well as is shown by the R-squared value. The R-squared value tells how well the model explains the variation in the willingness-to-pay. The model for the whole group (all) explained 33% of the variation in willingness to pay, the model for frequent fishers explained 43% of the variation and the non-frequent fishers 23%. Because angers willingness to pay is dependent on so many variables, including ones that cannot be measured in a survey, and it can often be difficult to fit a model to this type of data, these R-squared values show that the model fits the data relatively well (J.D. Snowball, Dept. of Economics, Rhodes University, pers comm. April 2006), however, other variables that affect the relationship should be investigated in order to strengthen further surveys that may take place. It should be noted that the model for the frequent fisher group fits the data better (that is, the determinants explain more of the variation in willingness to pay for frequent fishers), despite the smaller sample size, than for non-frequent fishers[4].

While a number of the variables of the complete model (all) were highly significant determinants of willingness to pay (significant at the 1% level), some of them had a very small effect on the WTP value, for example, on average, holding all other variables constant, one more trip a year increased mean willingness to pay bids by 0.1%. However, if respondents agreed that regulations were necessary, the average WTP increased by 34%. Similarly, being concerned about kob stocks increased average WTP by 16%. Demographics also had an influence on WTP responses and it was observed that having a high income job increased average WTP by 26% (discussed in the validity and reliability section – as economic theory would predict: a positive relationship between income and WTP bids being an indication that respondents were considering their budgets and giving realistic answers); while being of European-origin race group increased the average WTP by 43%[5].

When the sample is split between frequent and non-frequent fishers, some interesting differences emerge. In the frequent fisher group, knowledge of the status of the *A. japonicus* stock ("stocks" variable) increased the average WTP by 58%, and respondents who were of European-origin ("race" variable), had some tertiary education and were male, were willing to pay more on average for the programme (Table 5.4). Contrary to *a priori* expectations, frequent fishers who agreed that something should be done to facilitate stock recovery ("recovery" variable) had a 58% lower WTP than the mean. In the non-frequent fisher model, agreeing that regulations were important increased mean

[4] This indicates that other variables, not included in the model (because they were not measured), may be important in determining the willingness to pay for non-frequent fishers. For example, since 63 % of non-frequent fishers were not local to the Plettenberg Bay area, the costs incurred in travelling to Plettenberg Bay, their length of stay, environmental awareness, how important a part of their holiday fishing was amongst others might also be significant.

[5] In order to limit multicolinearity, where significant positive correlations were found, for example between education and job, the variable that provided the best fit was included.

WTP bids by 41%, in contrast to the frequent fishers for whom this variable was not statistically significant. The status of *A. japonicus* stocks and recovery variables were not significant determinants of WTP amongst non-frequent fishers, but being of European-origin and male significantly increased mean WTP bids (as in the frequent fishers group) (Table 5.4). Education was not a significant variable in determining WTP amongst non-frequent fishers.

5.4.2.1. Reasons to pay or not pay

Respondents were asked to give qualitative reasons for their willingness-to-pay responses. They were presented with numerous options and were asked to choose the one or few that best indicated the reason for their WPT responses. The questionnaire for those respondents that were willing to pay more and for those not willing to pay more is illustrated in Boxes 5.3 and 5.4, as well as in Appendix 1.

Box 5.3: The qualitative reasons given in the survey as to why respondents were willing to pay more for the recreational fishing permit.

Why are you willing to pay extra for a licence fee?

1. I may be catching more fish (personal gain)
2. I am concerned about conservation
3. I'll pay for a fishing licence no matter how much it costs (within reason) because I want to fish.
4. I like to support a good cause
5. I like to know that future generations will be able to catch kob

Box 5.4: The qualitative reasons given in the survey as to why respondents were not willing to pay more for the recreational fishing permit.

Why are you not willing to pay more than the existing R 60 for the fishing permit?

1. I don't have enough money
2. I don't believe the government will use the money for stock enhancement
3. I don't believe I will catch more fish, or that stock enhancement will work
4. I don't fish very much anyway
5. I don't target kob
6. It doesn't matter if the kob stocks become extinct
7. Don't support the idea of stock enhancement

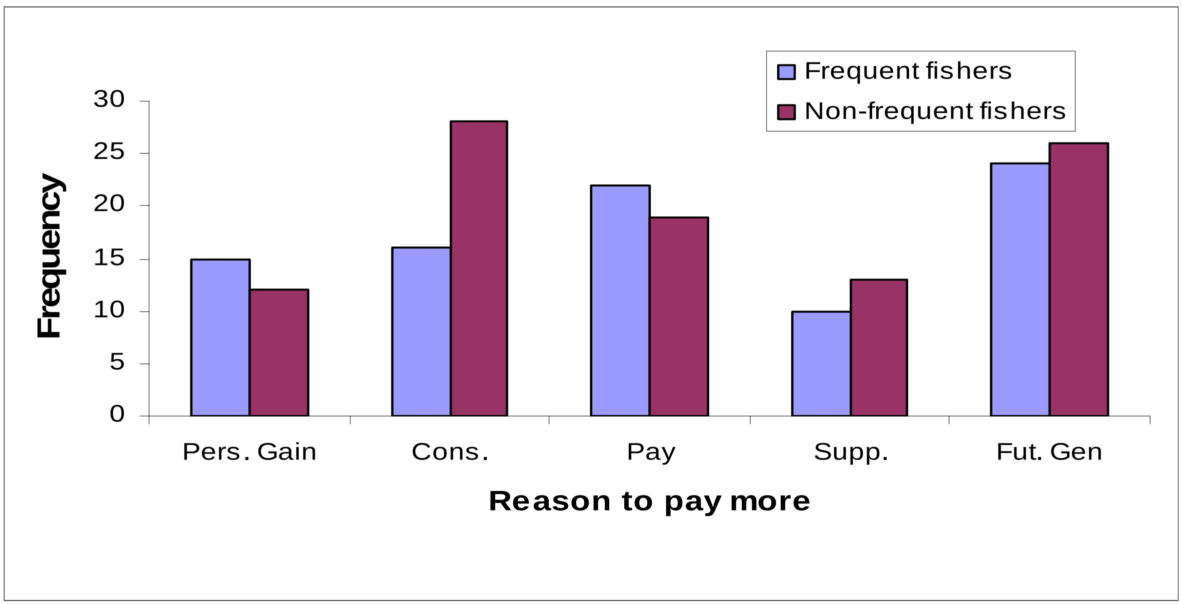

Figure 5.13: Shows the responses of the anglers to the reasons to pay an increased amount for a recreational fishing permit. (1) Pers. Gain: Personal gain, (2) Cons.: Concerned about conservation, (3) Pay: Pay because they enjoy fishing, (4) Supp.: Like to support a good cause, (5) Fut. Gen: Know that future generations will be able to catch kob.

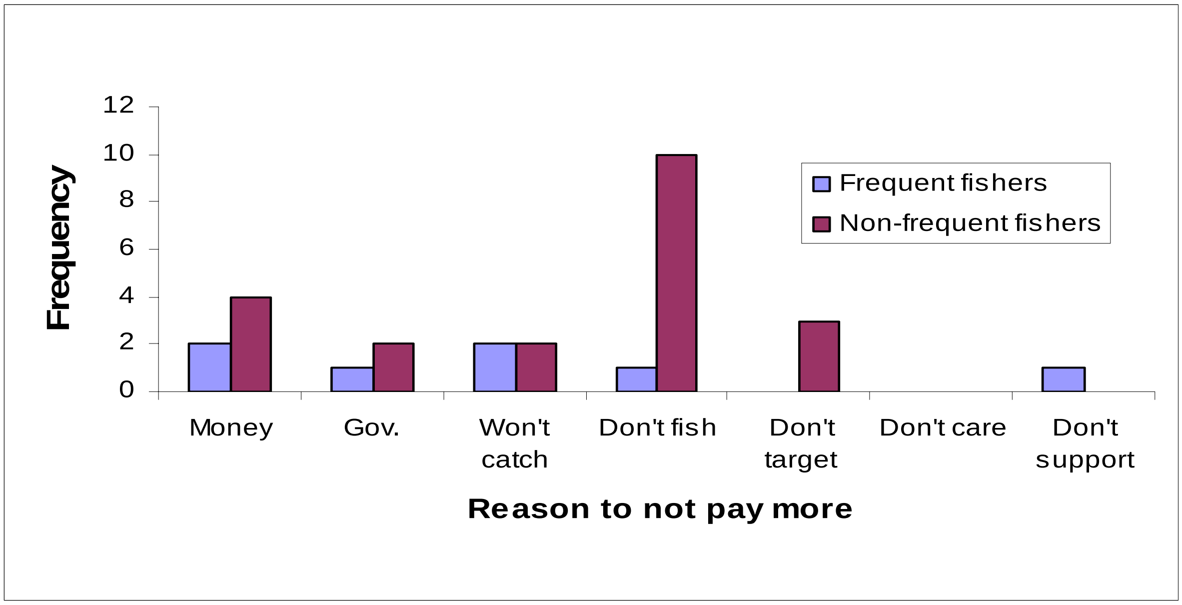

Figure 5.14: The responses of the anglers to the reasons as to why they were not willing to pay an increased amount for the recreational fishing permit. (1) Money: Do not have enough money, (2) Gov.: Do not believe the government will use the money toward stock enhancement, (3) Won't catch: Do not believe that they would catch more fish or that stock enhancement would work, (4) Don't fish: Do not fish very much anyway (5) Don't target: Do not target kob, (6) Don't care: It does not matter if kob become extinct, and (6) Don't support: Do not support the idea of stock enhancement.

A higher proportion of the non-frequent fishers cited their concern about conservation as a reason for their willingness to pay than the frequent fishers (Figure 5.13). The other options showed similar results between frequent and non-frequent fishers; however, personal gain and paying for the licence because of their love of fishing were slightly more prevalent among the frequent fishers. Not fishing very often was a major reason not to pay more among the non-frequent fishers (Figure 5.14). This appeared to be one of the major differences between the two groups and affected their willingness-to-pay results.

5.5. Discussion

5.5.1. Opinions, fishing habits and willingness to pay.

The opinions of anglers varied remarkably between those that fished frequently and those that fished non-frequently. Of the 102 survey respondents 43% were frequent fishers and 57% were non-frequent fishers. There were a higher percentage of non-frequent fishers that were not aware of the fishing regulations (size and daily bag limit). While 80% of all respondents agreed that regulations were necessary, this was higher for the frequent fisher group with a higher percentage of non-frequent fishers not knowing whether the regulations were important or not. This illustrates that frequent fishers were more concerned about the stock and showed a higher awareness of its status.

From the data, two distinct groups of anglers emerge: Frequent fishers who are mostly local residents with lower levels education and lower income, who go on about 93 trips per year spending an average of R 63 per trip, and non-frequent fishers who are mostly holiday makers with comparatively higher levels of education and income, who go on only about 8 fishing trips per year (no more than 25 trips per year) and spend an average of R 333 per trip (over 5 times that of frequent fishers). 66% of the frequent fishers were concerned about the status of *A. japonicus* stocks, as opposed to 58% of non-frequent fishers (Figure 5.9), possibly because frequent fishers are more reliant on these fish for subsistence purposes. The non-frequent fishers where concerned about the quality of their recreational experience rather than the number of fish that they actually caught. The data suggested that this may have been due to a lack of awareness of the status of the fish stock among the non-frequent fisher group (Figures 5.10 and 5.11).

An ongoing problem with the management of the recreational linefishery in South Africa is that of the lack of awareness and understanding of the purposes of the regulations and hence compliance with these regulations. This is apparent from the data collected in this study, with as high as 37% of the anglers encountered during the survey not having a permit. Furthermore, 89% of anglers reported that they had never, or hardly ever, reached their bag limit (Figure 5.12), suggesting that current bag limit regulations are not effectively protecting *A. japonicus* stocks in the Plettenberg Bay and Natures Valley areas. This is likely to be the case in the majority of areas along South Africa's coast as was illustrated by Griffiths (1997).

The frequent and non-frequent fisher groups that emerged not only differed in opinions but also in the amounts that they were willing to pay. Frequent fisher's median willingness to pay for the kob reseeding programme was R 155 per year, while that of the non-frequent fishers was R 100. As expected, this amount was lower than for that of the frequent fisher group, as these fishers do not use the resource as much as the frequent fishers. Statistically important determinants of frequent fishers willingness to pay were awareness that kob stocks are being depleted and demographic variables (race, sex, education), all of which were related to income level[6], while the statistically important determinants in the non-frequent group were whether or not they agree that recreational fishing regulations are important and their demographic variables, race and sex (Table 5.4). Because of their differences in opinion, it was different factors that influenced their willingness to pay for stock enhancement of *A. japonicus.*

The reasons for the responses of the anglers varied, but a pattern emerged, separating the frequent fishers from the non-frequent fishers, illustrated in Figures 5.14 and 5.15. The reasons that frequent fishers gave for paying an increased permit fee were dominated by the fact that they were concerned about their future generations being able to catch fish, that they would pay for the licence regardless of the price (within reason), as they needed a permit to fish and were not prepared to stop fishing because of not having a permit. Personal gain was a more influential factor amongst frequent fishers as they fish to catch fish to take home to eat, compared to the non-frequent fishers who fished primarily for the recreational experience. The non-frequent fisher's willingness to pay was mainly

[6] Contrary to *a priori* expectations, the frequent fishers who agreed that recovery was important and that "something should be done" were willing to pay less (although this variable is not highly statistically significant). A possible explanation is that, as the "stocks" variable indicates, it is frequent fishers who are most concerned about maintaining kob stocks. Since the frequent fisher group tended to have lower incomes, this may be translating into a lower average willingness to pay.

influenced by the fact that they were concerned about conservation, their future generations, and that they wanted to support a good cause. The non-frequent fishers were less concerned about how they would benefit from a stock enhancement programme, but rather how the environment would benefit. This is likely to be because they do not use the resource as frequently and would not benefit as much in terms of personal gain from catching more fish on each fish trip. This scenario is tending towards non-market value. This is when respondents are willing to pay in order to keep the species from becoming extinct. They want the species to exist even if they don't know that that they may benefit from the existence of the species (Knight and Bates, 1995).

For 18% of the frequent fishers who were not willing to pay more, the reason was mainly that they could not afford to pay more than what they were currently paying, while 36% of the non-frequent fishers argued that they did not fish frequently enough to warrant paying more (Figure 5.14). Although the frequent fisher group consisted of a larger proportion of respondents from the lower income group, these anglers were willing to pay on the grounds that they fished often and would pay the licence fee because they needed the licence to fish.

5.5.2. Cost-benefit analysis

It is acknowledged that the small sample (102 anglers) in a restricted area is not representative of the national population of anglers in South Africa and that by simply extrapolating the WTP results to the national population would yield inaccurate results. However in a more comprehensive study with a larger sample size extending over larger area, the results could be extrapolated more accurately in a method similar to that used in the following cost benefit analysis.

An accurate cost benefit analysis is constrained by the lack of information on the number of fish required for stock rebuilding and the survival rate of stocked juveniles. However, based on the WTP survey it is possible estimate potential income to finance stock enhancement from angling licences, and the costs of producing hatchery reared fish are known (Chapter 6). It is thus possible to model the costs of various stocking strategies.

According to the Department of Environmental Affairs and Tourism: Chief Directorate Marine and Coastal Management (2005), there are approximately 450 000 recreational angling permit holders in South Africa annually (this does not include other sectors, for example bait collecting and rock lobster permits). However, as the licence fee increases, so the number of licences bought is likely to decline (the law of demand: as price rises, quantity demanded falls). Figures 5.15a and 5.15b show the trade-offs between anglers' willingness-to-pay for the recreational fishing permits and the funds generated for both frequent and non frequent fishers.

The first objective of the draft traditional linefish management policy is to increase the participation in the fishery by black traditional line-fishers (Department of Environmental Affairs and Tourism Chief Directorate: Marine and Coastal Management, 2005). Therefore, the highest income option is not necessarily the most appropriate, as policy in South Africa aims to provide affordable access to the resources which should not be for the exclusive use of high income earners.

As expected (Carson *et al*. 2000), an increase from the current amount/cost of a permit (R 60) will result in a decrease in the number of anglers willing to purchase a permit (Figure 5.15a and 5.15b); however, this cannot be avoided if fishing permits are to be the vehicle of payment for stock enhancement.

In the cost benefit analysis shown in Figures 5.15a and 5.15b, the maximum willingness-to-pay value was the upper limit that anglers would pay for a fishing permit, and if the permit was increased further then the angler would not pay for the licence. So using the anglers maximum willingness-to-pay as a proxy for 'no longer purchasing a licence,' an increase from R 60 to R 70 would result in a 32% decrease for non-frequent fishers and a 16% decrease for frequent fishers, while a further increase from R 70 to R 100 would result in only a further 1% and 3% decrease in the number of anglers willing to pay for a recreational fishing permit for the respective groups. There after, a substantial drop is observed, resulting in exclusion of up to 50% of anglers from the recreational linefishery, even if the licence is increased to R 110. This initial 32% and 16% drop-off with a R 10 increase in the permit price is likely to be an overestimate. This large initial drop-off with subsequent marginal change is reflects the typical objection vote to change. Basically anglers are saying that they will not pay more in the hope that if they say that then the fee will not increase. Although this biases willingness to pay results, it does so in a negative direction, and at worst will provide conservative results

Although it is inaccurate to suggest that all anglers fit into a single group, either the frequent fisher group or all fit into the non-frequent fisher group, this is done purely to eliminate the effects of avidity bias. And although the accuracy of the estimation may be compromised, a conservative estimate is obtained. The actual amount generated will be somewhere in between the values produced by each group, depending on the actual proportion of frequent and non frequent anglers.

Using the results displayed in Figure 5.15a and 5.15b, it was calculated that an increase in the licence fee to R 100 would generate additional annual funds of approximately R 14 720 000 if all licensed anglers were frequent fishers, and R 11 500 000 if all licensed anglers were non-frequent fishers – values both which are substantially higher than that estimated to be required for the stock enhancement programme, even in the start-up year (see Chapter 6).

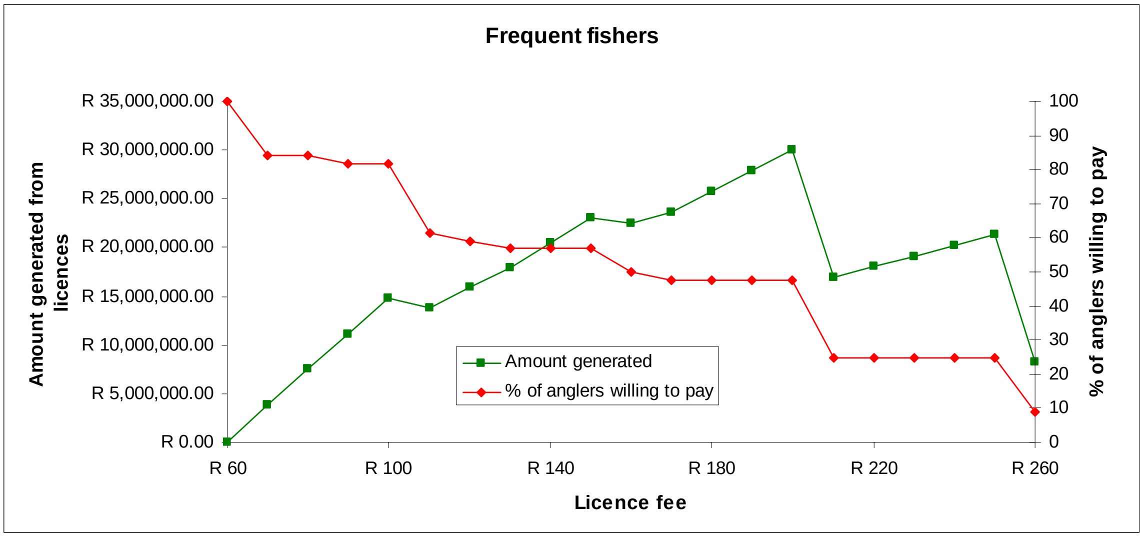

Figure 5.15a: Cost benefit of increased licences for frequent fishers (assuming all 450 000 licensed anglers are frequent fishers), as well as percentage of anglers willing to pay the increased licence fee.

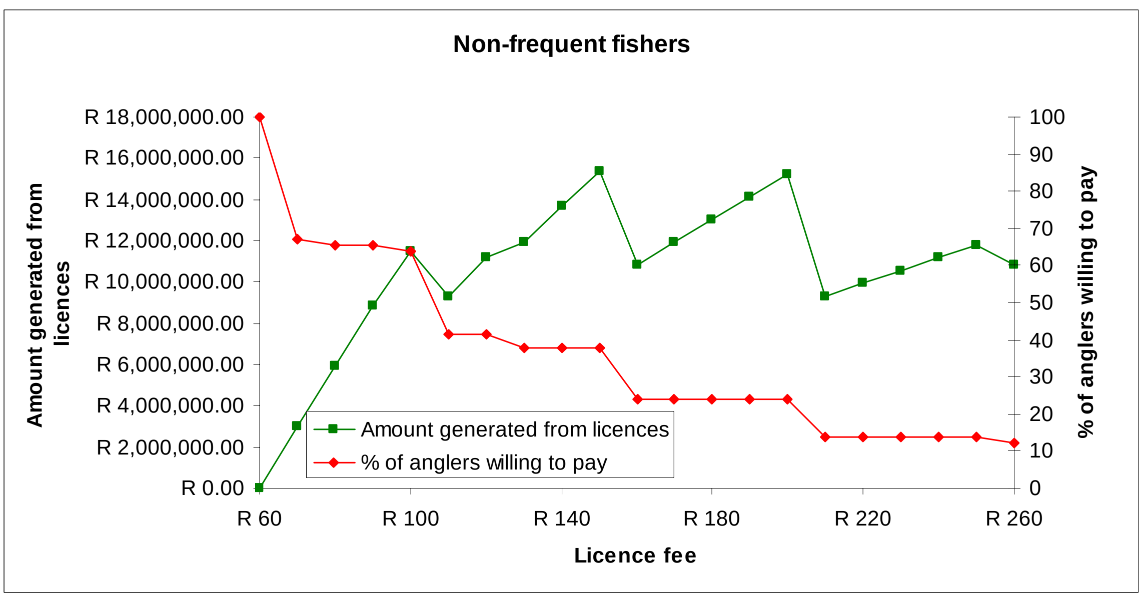

Figure 5.15b: Cost benefit of increased licences for non-frequent fishers (assuming all 450 000 licensed anglers are non-frequent fishers), as well as percentage of anglers willing to pay the increased licence fee.

5.5.3. Validity and reliability of the WTP survey

The biases associated with the willingness-to-pay method are discussed in this chapter and it is recognised that with careful questionnaire design, and data analysis, these biases can reduced, thereby producing more reliable and valid information. To test that this was achieved in this survey validity and reliability tests were performed.

Validity refers to the correspondence between what one wishes to measure and what one actually measures, while reliability refers to the replicability of the measurement (Carson *et al*. 2000). The ideal way in which to determine validity is to compare the measurements made with some reference or criterion measurement that is known to be correct. However, no such reference or criterion exists to which contingent valuation WTP measurements can be compared. This is similarly the case with any consumer surplus estimate, regardless of the econometric technique used (Carson *et al.* 2000). Consumer surplus represents the difference between what the respondent paid and the maximum amount that they would have paid, a value which is unobservable (Carson *et al*. 2000).

Carson *et al*. (2000) identify several tests of validity and reliability for judging the success of a WTP survey. The first of these is construct validity, which tests the extent to which the findings are consistent with predicted theoretical expectations.

Construct validity tests were conducted on the data collected in this study to assess the validity of the survey. It was expected that WTP should be positively related to income. Since income data could not be obtained directly, race has been used as a proxy for income. This is deduced from the level of education and job type data shown in Figures 5.5 and 5.6. As expected, there is a statistically highly significant positive relationship between race and WTP and shows that being of European origin increases the mean WTP by 43% (Table 5.4). Similarly those who use the resource frequently are expected to be willing to pay more than only use it occasionally (Carson *et al*. 2000). It was observed in this study that frequent fishers had a median WTP more than 50% higher than that of the non-frequent fishers. The results of these qualitative tests therefore lend support to the validity of the construct of the study.

Tests of reliability require the replication of the study at another time (temporal reliability) or with another sample population (geographical reliability) (Carson *et al*., 2000). Given that construct validity of the survey has been shown, it is suggested that it would certainly be worth extending this pilot study to test the reliability of the survey instrument. However, it was beyond the scope of the present study to undertake further survey work.

5.6. Conclusion

The findings of this willingness to pay survey, conducted using face-to-face interviews with 102 fishers between Plettenberg Bay and Nature's Valley show that there are definite differences in opinion regarding the importance of fishing regulations and concern over *A. japonicus* stocks between frequent and non-frequent fishers. Despite having lower average income and education levels, it is the frequent fishers who are most aware and concerned about kob stocks and who are willing to pay more for the proposed stock enhancement programme.

It was also shown that the majority (75%) of anglers were willing to pay more than the current R 60 for a recreational angling permit. With 450 000 recreational anglers in South Africa, even a minimal increase in the cost of an angling permit equates to a substantial amount of money. From the small sample of 102 permitted anglers limited to the Plettenberg Bay and Natures Valley area, this study showed that recreational angling permits may be increased to as high as R 100 without excluding an unreasonable number of anglers from the fishery. A larger scale study is, however, required before any reasonable assumptions about the amount of money generated can be made, but is likely to be of the order of R10 000 000 annually.

Although the funds generated by increasing the price of the permit, may not all be made available for stock enhancement, as other linefish depend on the same source of funding, there is still a substantial amount that could be made available. The economic feasibility of stock enhancement of *A. japonicus* should not be viewed as a fatal flaw and the decision to use it as a management tool should not be influenced by this.

Stock enhancement of *A. japonicus* deserves further investigation as a management tool and should not be overlooked on the basis of its costs, as it has been shown in this study that there is potential to fund such a management tool through increased recreational fishing permit costs, without excessively decreasing the annual number of permits purchased.

Construct validity tests suggest that the results of the survey are valid, and can be successfully used as a tool to collect data on anglers' willingness to pay for a stock enhancement programme. It is recommended that this pilot study be extended to include other sample populations and other times to confirm its temporal and geographic reliability.

CHAPTER 6

AQUACULTURE OF *ARGYROSOMUS JAPONICUS* FOR THE PURPOSE OF STOCK ENHANCEMENT IN SOUTH AFRICA

6.1. Introduction

The ability to culture a species in captivity, in a cost effective manner, ultimately determines the potential for the use of stock enhancement as a management tool for that species (Molony *et al.* 2003). This chapter investigates the economic feasibility of stock enhancement of *A. japonicus* using the data collected in the willingness-to-pay survey (Chapter 5), and a hatchery costing model.

The aquaculture industry has developed rapidly over the past 50 years (Westers 1986), mostly as a result of spin-offs from the rapid technological advances in other fields. This has resulted in the capability to culture a rapidly increasing number of species of fish, including many for stock enhancement purposes.

Although not a leading aquaculture producer, South Africa is no exception when it comes to the growth and development of aquaculture, with the sector showing a 663% growth in production between 1982 and 1992 (Chimatiro 1998). Aquaculture in South Africa consists mainly of freshwater species such as trout, catfish and tilapia, and marine molluscs such as abalone, mussels and oysters. However, marine finfish such as *A. japonicus*, *Pomadasys commersonnii*, *Seriola lalandi* and tuna species have more recently been investigated (Brink 2003), opening up possibilities for stock enhancement.

6.1.1. Aquaculture of *Argyrosomus japonicus*

Argyrosomus japonicus is being developed as an aquaculture species both in Australia and South Africa. The species makes a good candidate for aquaculture as it grows quickly (reaching market size in a year), is tolerable of a wide range of environmental conditions, forms slow moving shoals meaning that they can be reared at relatively high stocking densities, and they show efficient food conversion ratios of between 1.0:1 and 1.2:1 (Oellermann 2000). The artificial induction of spawning through the use of hormones has been carried out successfully in both Australia and South Africa, and the technology and the capability to rear the species in captivity to a marketable size, or a size suitable for re-stocking, now exists.

6.1.2. Commercial aquaculture vs stock enhancement

Several fundamental differences exist between aquaculture for commercial purposes and for that of stock enhancement. These differences are identified in chapters 2 and 3. It is important to keep this in mind when designing a hatchery for stock enhancement and it has implications for the setup and running costs of such a hatchery.

6.2. Methods

6.2.1. Costing model

The economic feasibility of the stock enhancement of *A. japonicus* in South Africa is ultimately determined by the amount of money generated by the increase of the price of recreational fishing permits, and the costs associated with the production of juveniles for release. Chapter 5 investigated anglers' willingness to pay for recreational fishing permits and concluded that it was possible to generate over R 12 million by increasing the price of fishing permits to R 100. In this chapter a model was created to estimate the setup and running costs of a hatchery for the stock enhancement of *A. japonicus* in South Africa. Several parameters were estimated (Boxes 1 to 8) and were used to calculate costs involved in setup and running of a stock enhancement hatchery for *A. japonicus.* These parameters include biological parameters and system requirements, feeding regimes, biological filtration, tank sizes, pumping requirements and tagging costs.

6.2.2. Recirculation system

One of the most important requirements in the aquaculture of *A. japonicus* is that of clean and chemically stable seawater. An efficient method of maintaining stable water conditions is to employ a recirculation system in the hatchery and grow-out facilities. A recirculation system allows for manipulation of temperature to optimum levels, it reduces pumping costs and it can be shut off from the sea during high risk events such as red tides or pollution spills.

Although *A. japonicus* are tolerant of a wide range of water quality parameter, the best growth and survival rates occur when the fish are kept at their optimal conditions (Table 6.1).

Table 6.1: Optimal water requirements for *A. japonicus* aquaculture (SA Aquaculture 2003).

Temperature:	20 °C
Temperature range:	18 °C – 25 °C
O_2:	5 $mg.L^{-1}$
Salinity:	12 ‰
Salinity range:	10 ‰ – 40 ‰
pH:	8.1
Ammonia:	≤ 0.1 $mg.L^{-1}$

The technology required for the production of *A. japonicus* for enhancement, which is a function of the biological requirements of the species, is a major factor in determining the economic feasibility of the stocking programme, and therefore has to be considered when producing a costing model for the stock enhancement. The costing model considers the requirements at all levels; from the setup of the hatchery, through to broodstock maintenance and the growout of juveniles for release. The use of a recirculation system also allows for far greater flexibility in terms of the site for the hatchery and allows for a more accurate estimation of land cost.

The key biological characteristics that determine the setup and running costs of a hatchery associated are listed in Box 6.1. Larval survival determines the number of larvae required to produce a given number of post larval fish, and similarly, fingerling survival determines the number required to produce a given number of fish of suitable size for release. This affects hatchery costs as it influences the number of fish that are fed prior to release that are not ever released. Mortality rates decrease as the fish develop from larval to post larval stages. These survival rates are given in Box 6.1. Size at hatch of the larvae and growth rate allow for a growth curve to be created on which feeding costs and tank requirements can be based. The maximum stocking density determines the number of tanks required for the production of a given number of fish.

Box 6. 1: The Biological parameters used in the costing model (Aquaculture SA 2003).

Biological parameters	
Max. size of larvae (mm)	26
larval survival (%)	15
fingerling survival (%)	60
size at hatch (mm)	2.3
growth (mm.day^{-1})	0.8
maximum stocking density (kg.m^{-3})	50

The feeding regime applied in the hatchery affects the production costs of fish as the different diets required at different stages of development differ in price. The diet used in the model is shown in Box 6.2 and is also described in the larval rearing and growout section of this chapter. Similarly enrichment of livefood adds additional cost to the feeding with livefood, in this case rotifers and artemia.

Box 6.2: The feeding regime used to estimate feeding costs in the costing model.

Feeding	**Rotifers**	**Artemia**	**Pellets**
start feeding (day)	3	8	25
end feeding (day)	13	30	Stock-out
cost of food (R.kg^{-1})	50	1000	10
feed rate (indiv.L^{-1}) (%BW.day^{-1})	5000	5000	3

Box 6.3: The enrichment of food used n the costing model.

Enrichment	
Enrichment (Rand.kg^{-1})	800
Number of fish that can be fed 1kg enrichment	300000

Box 6.4: The size of the tanks used in the costing model.

Tanks	**Rearing**	**Broodstock**
size of tanks (m^3)	5	10

The biological filter parameters affect the setup costs as the cost of construction of a biological filter is dependent on the size of that filter. In this case the filter size is determined by the amount of food fed in the system, the conversion rate of ammonia to nitrate of the biological filter and the surface are of the filter medium (Box 6.5).

Box 6.5: The biological filter parameters used in the costing model

Biological filter	
surface area to volume (m^2.m^{-3})	250
1m^2 converts ammonia to nitrate at (g.m^{-2}.day^{-1})	0.3
kg ammonia released from kg food (50% protein)	0.032

The selection of broodstock should be done in accordance with the genetic requirements discussed in chapter 3, based on sound genetic studies. The parameters chosen here were used to estimate the costs of the hatchery. Conservative figures (rather too high than too low) were used for broodstock number and size in order to not underestimate the setup and running costs of the hatchery.

Box 6.6: The broodstock used in the costing model

Broodstock	
Number	150
Size (kg)	12
broodstock density (kg.m^{-3})	12
tank size (m^3)	10
Feeding rate (%BW.day^{-1})	3

Box 6.7: Estimate of tagging cost used in the costing model

Tagging	
Post release monitoring (R)	300000
Cost of tagging per fish (R)	0.00075

Pumping costs were estimated based on the basic water requirements for the recirculation system of the hatchery.

Box 6.8: Pumping requirements used as a basis for costs in the costing model.

Pumping	
Water exchange rate (% per day)	10
Broodstock tanks ($L.day^{-1}$)	15000
Rearing tanks ($L.day^{-1}$)	50357
Total volume per day (L)	65357
Pump size (kW)	1
Electricity price($Rand.kW^{-1}.h$)	0.4

Using the parameters shown in Boxes 6.1 to 6.8, both setup and running costs were calculated based on estimates for varying numbers of fish of lengths ranging from 25 mm to 125 mm. The setup costs were based on the following:

- Site and Building
  - This is a fixed cost as the land and basic facility cost does not change with production. The change in building size with production is catered for by the additional building costs.
- Additional building
  - This additional building cost takes into account for extra building space required to house fish according to the number and size of fish produced.
- Pump house
- Algae and livefood room equipment
- Alarm system
- Air conditioning
- Air supply

- Larval system
  - The cost of the larval rearing system varied with the number of fish being produced. Biological requirements were taken into account when estimating the cost for a given number of fish.
- Broodstock system
  - The broodstock system was based on a broodstock size of 150 individuals with an average of 12 kg each.
- Algal ponds
- Generator
- Vehicles

While the running costs of the hatchery were based on the following:

- Fish production
  - A fish production cost were based on the food for broodstock and juveniles, enrichment of livefood and tagging with OTC, and was dependent on the number and size of fish for release.
- Depreciation of the facility
  - Depreciation was estimated to be 8% of the total setup cost per year.
- Electricity
- Salaries
- Pharmaceutics
- Administration
- Maintenance
- Transport
- Miscellaneous
  - The miscellaneous costs were estimated to be 5% of the overall running costs.

6.3. Results

The cost of stock enhancement of *A. japonicus* varies according to the number of fish released and the size of the fish released (Figure 6.1 and 6.2). Both setup and running costs increase with an increasing number of fish produced as well as with the release of larger fish. This is due to requirements of pumping, tank space and feeding costs. Figure 6.1 shows that with the parameters used in Boxes 6.1 to 6.8, the setup costs of a hatchery for *A. japonicus* would range between R 10 000 000 and R 30 000 000 depending on the number and size of fish released. Similarly, Figure 6.2 shows that the running costs of the hatchery would range between about R 2 600 000 and R 6 700 000 per year. The number of fish produced varies between 100 000 and 5 000 000 fish of size 50 mm to 150 mm.

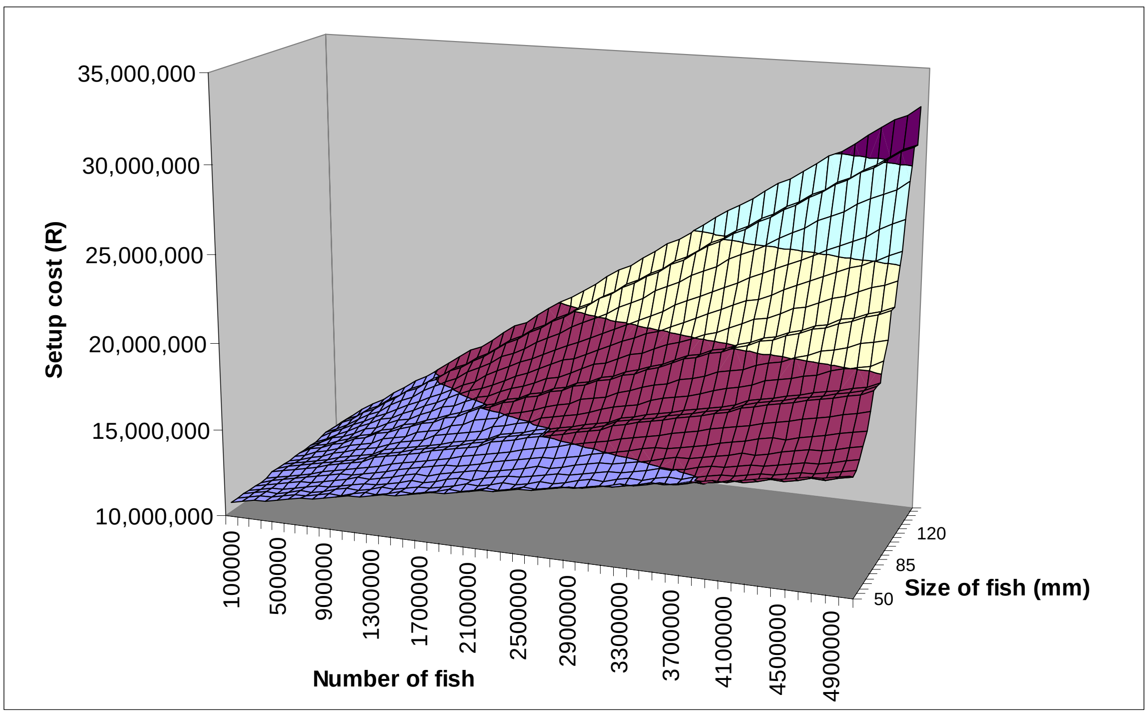

Figure 6.1: Setup cost of a hatchery 100 000 to 5 000 000 fish of between 50 mm and 150 mm.

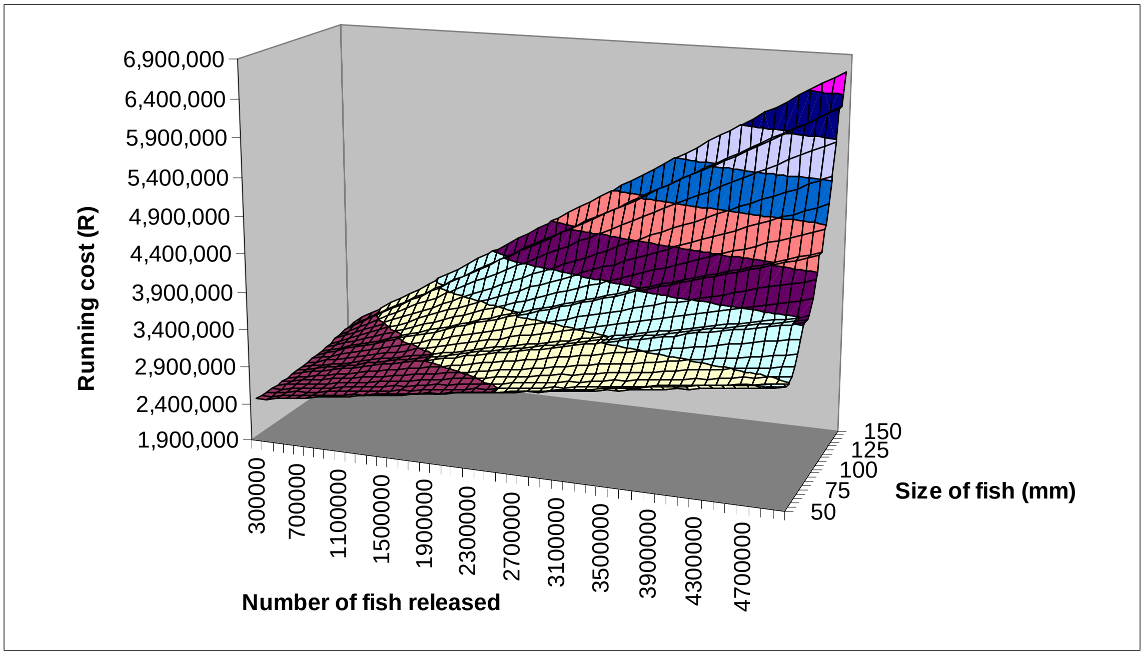

Figure 6.2: Annual running costs for hatchery to produce varying numbers of fish for release at different sizes.

Tables 6.2 and 6.3 show how the costs for setup and running of the hatchery were calculated. This was done for a series of values of number of fish and size at release to produce the graphs in Figures 6.1 and 6.2.

Table 6.2: An example of capital costs for a hatchery to produce 1 000 000 juvenile *A. japonicus* of 100 mm in length.

Capital costs	
Site and Building	R 8,000,000.00
Additional building (production dependant)	R 812,500.00
Pump house	R 200,000.00
Algae & livefood room equipment	R 50,000.00
Alarm system	R 4,000.00
Air-conditioning	R 130,000.00
Air supply	R 180,000.00
Larval system	R 2,560,000.00
Broodstock system	R 760,000.00
Algal ponds	R 20,000.00
Generator 350kW	R 180,000.00
Vehicles	R 300,000.00
Total capital	**R 13,196,500.00**

Table 6.3: An example of the running costs to produce 1 000 000 juvenile *A. japonicus* of 100 mm in length.

Running costs pa	
Fish production	R 405,280.36
Depreciation (8% pa)	R 1,055,720.00
Electricity	R 100,000.00
Salaries	R 800,000.00
Pharmaceuticals	R 12,000.00
Administration	R 30,000.00
Maintenance and repair	R 12,000.00
Transport	R 10,000.00
Miscellaneous (5%)	R 121,250.02
Total	**R 2,415,000.36**

6.4. Discussion

6.4.1. Land and building

The entire setup cost and a substantial proportion of the running cost comes from the setup and maintenance of the hatchery facility (Langton and Wilson 1998). The setup costs include those items shown in the example in Table 6.2, while the maintenance of the hatchery is represented by 8% depreciation as illustrated by the example given in Table 3.

The land and setup of a basic hatchery was estimated to be R 8 000 000, which is not a highly variable figure that is dependent on the location of the site, because a recirculation system is less site dependent than a flow-through system. Additional building costs were than added according to the number and size of fish being produced and the subsequent hatchery size required to house the additional tanks. The prices used to estimate setup costs of a hatchery for stock enhancement were based on an unpublished business plan for the production of juvenile *A. japonicus* by Van Rooyen *et al*. (2004); however, costs were adjusted for the number and size of fish being produced and the subsequent size of the building required (Figure 6.1)

6.4.2. Broodstock

The capture and maintenance of broodstock can be a significant expense for a stock enhancement programme (Langton and Wilson 1998). This is particularly relevant for *A. japonicus*, because coupled with the large numbers of broodstock fish required, mature *A. japonicus* are large (maturing at > 100cm) and therefore require large quantities of food and large volumes of water. This influences both the setup, in terms of holding space, and running costs of the hatchery.

The importance of maintaining genetic diversity is discussed in detail in chapter 3 of this study. In terms of costs, the main implication of the genetic management is the large numbers of broodstock required (Cross 1999, Moore and Seeb). The costing model in this study based costs on a broodstock of 150 individuals of 12 kg each which are fed a pelleted diet at 3% body weight per day (Box 6.6). This is a conservative value but allows for room to have such a large broodstock if research shows that such a large number of individuals are in fact required. Compared to commercial *A. japonicus* producing aquaculture facilities the cost of broodstock maintenance is substantially higher, as commercial facilities may have as few as eight individuals to produce the same number of juveniles (Dr. Niall Vine, Dept. Ichthyology and Fisheries Science, Rhodes University, pers. comm. March 2007).

The variables that affect the setup and running costs of the hatchery include the stocking density, size of the tanks and feeding rate. The values used for each of those variables are shown in Box 6.6, and were set at values as realistic as possible and are based on those

6.4.3. Spawning

The management of genetic diversity in a stock enhancement requires the careful control of the spawning of individual fish. In order to have control over spawning of individuals, one needs the ability to induce spawning through the use of hormones or environmental cues, which has been achieved with *A. japonicus* in Australia in 1992 (Battaglene and Talbot 1994). Battaglene and Talbot (1994) reported to have injected *A. japonicus* with 250 IU and 1000 IU of human chrorionic gonadotropin per kg of body weight, for males and females respectively, and the fish were successfully strip spawned 32 hours after the injection. More recently the use of GNRHa has become common and successful, with several commercial brands being available (Dr. Niall Vine, Dept. Ichthyology and Fisheries Science, Rhodes University, pers comm. 2007). This gives managers the ability to strip spawn individuals, and provides good opportunity for careful hatchery management in terms of the maintenance of genetic diversity of fish for release (Chapter 3). This had implications for the costing model, because to have a strict control over the fish, they should be kept in relatively small tanks, where individuals could be monitored. Pharmaceutical costs also had to be taken into consideration as hormones are required to induce spawning.

6.4.4. Larval rearing and growout

A large proportion of the cost of stock enhancement lies within the larval rearing and growout of juvenile fish to the appropriate size for release. The major factors that influence the cost of larval rearing and growout are diet, stocking density, and size at release. These factors influence the cost of having juveniles in the hatchery and the time that that they spend in the hatchery.

For the purpose of this study and to make realistic estimations of costs, the diet regime described below was used as a basis for costs. Juvenile *A. japonicus* hatch at about 2.3mm in length and from the third day after hatching are capable of feeding on a diet of enriched rotifers. This diet is continued until 13 days after hatching. From day 8, the juveniles are weaned onto enriched brine shrimp (*Artemia sp.*), and that diet is continued until day 30. Once the juvenile *A. japonicus* reach 25 days old, a diet of artificial pellets is suitable and this diet can be continued until the juveniles reach a suitable size for release. In growout aquaculture, feeding costs form a much larger proportion of the running costs, but as these fish are small and are only kept in the hatchery for a short time, feeding costs are not as high as in I growout facility.

A. japonicus naturally form large shoals and therefore stocking density is dependent on water quality parameters rather than fish behavioural restrictions. A stocking density of 50 $kg.m^{-3}$ is suggested to be adequate for juvenile *A. japonicus* (SA Aquaculture 2003) and this is what the costing model is based upon. The stocking density has obvious effects on the number of tanks required to hold the juvenile fish which substantially affects the setup costs of the hatchery.

6.4.5. Tagging

Aquaculture for the purposes of stock enhancement differs from that of commercial aquaculture in that emphasis is no longer placed on the most cost effective production of fish but rather on the genetic and ecological qualities of the fish being produced (Chapters 2 and 3). Chapters 2, 3 and 4 discuss the importance of post release monitoring and chapter 4 identifies the use of Oxytetracycline-HCL for identification of hatchery reared fish after release. Tagging costs were included in the costing model, but fortunately, OTC is a very cost effective method of tagging large numbers of fish and does not contribute a large amount to the overall running cost of the hatchery (Box 6.7).

6.4.6. Number of fish vs size at release

The rate of natural mortality, among fish in the wild, decreases as their size increases. Therefore, the longer fish are kept in the hatchery, where an unnaturally high mortality is maintained, the higher the survival among the released fish. The resulting decrease in natural mortality among juvenile fish can cause ecological and genetic problems to occur (Chapters 2 and 3). To release smaller fish would have less of a genetic and ecological effects, however, would reduce the effectiveness of stocking, unless large numbers of fish were released. Figures 6.1 and 6.2 show that production and running costs increase more rapidly with the size of the fish than the number of fish being released. It is therefore more desirable, both ecologically and economically to release a large number of smaller fish. However, due to handling stress, it is not possible to handle and transport juvenile *A. japonicus* smaller than 25 mm to 30 mm TL (Guy Musson, Espandon Marine, pers. comm. January 2007).

6.5. Economic feasibility of stock enhancement of *A. japonicus* in South Africa

The figures in this study show that setup costs for a stock enhancement orientated hatchery would be in the range of R 10 000 000 and R 30 000 000, while annually running costs would be between R 2 000 000 and R 6 000 000, depending on the number and size of fish produced. The promising results, obtained in the willingness-to-pay survey suggest that a substantial amount of money could be generated by increasing the price of the recreational fishing permit. Although larger scale studies are required to estimate the exact amount that could be generated and management decisions made as to how much would be made available to stock enhancement of *A. japonicus*, it is likely that the costs could be covered by the increased licence fee in a period of just a few years.

CHAPTER 7

CONCLUDING DISCUSSION

7.1. Introduction

At present the spawner biomass per recruit of *A. japonicus*, a popular species among recreational anglers, is estimated to be between 1% and 4.5% of pristine, the fishery has collapsed and the species is listed as vulnerable in South Africa. Current management procedures (based on traditional size and daily bag limits) are not working effectively to facilitate the recovery of the species (Griffiths 1997a). The reason that current management is not facilitating recovery is that compliance is poor among resource users, and that the regulations are such that they do not facilitate recovery. In order to make regulations effective, the *A. japonicus* fishery would need to be closed until stocks have recovered, or the size limit increased. This would require increased enforcement of compliance, which would require increased funding. It is unlikely that funds would be made available through the users, because the *A. japonicus* would no longer be allowed to be caught, and anglers would not be willing to "pay more and get less". Intervention in the form of alternative management is, therefore, required to help facilitate the recovery of *A. japonicus*. Stock enhancement was, therefore, investigated as a possible tool to be used in conjunction with traditional management measures to actively facilitate the recovery of the stocks.

Bell (2004) suggests that stock enhancement should only be considered when wild production is recruitment limited, and when it is predicted that other management tools will result in unreasonably long time periods to facilitate the recovery of stocks. It is not certain whether *A. japonicus* is indeed recruit limited, but given its life history strategy, late maturity at large size, it is likely that it may well be. However, further research is required, and if this reveals that stocks are indeed recruit limited, then stock enhancement is a management tool that should be considered. Available evidence indicates that the current management of *A. japonicus* is not sufficient to facilitate the recovery of the species in a reasonable time frame, with Griffiths (1997a) suggesting that it could take over 40 years for stocks to recover.

Although no marine stock enhancement has being undertaken in South Africa, a draft policy exists regarding legislation pertaining to stock enhancement. This policy requires that a strict scientific approach be taken towards stock enhancement as it recognises the environmental risks of such an activity.

This study investigated the technical and economic feasibility of stock enhancement of *A. japonicus* in South Africa, as well as the possible risks involved and methods of minimising these dangers. The areas covered in this study were those in which any obvious fatal flaws, which would eliminate the possibility of using stock enhancement for rebuilding *A. japonicus* stocks, would immediately become apparent.

7.2. Ecology

By stocking hatchery reared fish, an ecosystem is immediately affected in some way. With the correct information, negative ecological effects can be minimised and kept at acceptable levels. Before stock enhancement can be undertaken, a better understanding of the size and shape of the *A. japonicus* stock in South Africa is required, and based on this information; guidelines for optimal release strategies can be formulated. Research is required on the selection of suitable release sites, estimation of the carrying capacity, particularly for the envisaged releases sites, competition effects, and disease control of hatchery reared *A. japonicus*. These aspects of the ecology of stock enhancement of *A. japonicus* are eminently achievable given the available scientific capacity.

7.3. Genetics

Any programme of stock enhancement requires an active genetic management programme to ensure that the genetic profile of the wild population is not altered due to selective breeding and the unnaturally high survival of juveniles from a limited broodstock pool.

Genetic analysis methods are available to assess firstly, the genetic structure of wild *A. japonicus* stocks, and secondly, the genetic effects on a hatchery reared population intended for release. It appears that MS-DNA analysis would be the most appropriate for these types of studies and at the time of writing a study on kob population genetics was underway based at the University of Pretoria.

Methods of minimising the risk of genetic problems were highlighted in Chapter 3, and should be incorporated into management plans. These include strategies such as releasing fish at a smaller size, rearing fish under natural conditions and maintaining a large broodstock pool representative of the wild genetic profile.

7.4. Tagging

The importance of post-release monitoring is essential for the evaluation of the effectiveness of the stocking programme as well as for assessing the ecological end genetic effects of stocking. This project assessed the suitability of coded wire tags, visual implant fluorescent elastomers and oxytetracycline-HCL, based on ease of use, effects on growth, and tag retention in juvenile *A. japonicus*. OTC appeared to be the most suitable method for use in *A. japonicus* as it was easily applied to a large number of fish, did not affect the growth rate, and showed 100% retention compared to the 61% and 62% of CWT and VIFE respectively. OTC is, therefore, suggested for post release monitoring in pilot and full-scale studies of stock enhancement of *A. japonicus.*

This provides the potential for effective post-release monitoring of *A. japonicus*, which is an essential requirement for a responsible stock enhancement programme. With an effective tagging method, genetic and ecological effects of stocking can be monitored, as can dispersal, survival and growth rates. This will provide the required data for the determination of economic feasibility.

7.5. Willingness to pay

The willingness-to-pay for stock enhancement was tested in a pilot survey among 102 recreational anglers in the Plettenberg Bay and Natures Valley areas. The results were positive indicating that in general, anglers would be willing to pay more for their permits if kob stocks were enhanced. The willingness-to-pay method proved to be an effective tool which can be used on a larger scale to more accurately determine the economic feasibility of stock enhancement. A larger scale study, including anglers from a wider distribution, should be conducted using the willingness-to-pay method.

With a recreational linefishery of comprised of approximately 450 000 anglers, an increase in the cost of the fishing permit has the potential to generate a substantial income. Although conclusions, regarding the amount of money that could be generated, cannot be drawn from this study (due to its small sample size and restricted distribution along the coast) it can be estimated that in the vicinity of R 10 000 000 may be generated for the MLRF.

The potential to generate substantial funds which could be used towards the stock enhancement of *A. japonicus* does exist. Economic feasibility does, therefore, not appear to be a limiting factor in the decision of whether to use stock enhancement or not. The decision can rather be based on management needs and any ecological or genetic issues that may arise in further studies.

7.6. Aquaculture

Capacity for the commercial production *A. japonicus* in captivity is now established in South Africa, making stock enhancement a technically feasible option for the management of *A. japonicus* in South Africa.

A costing model was, therefore, developed based on known parameters from the existing industry, and the requirements specific to a stock enhancement programme. The model showed that initial setup costs of a hatchery for the production of juvenile *A. japonicus* would range between R 10 000 000 and R 30 000 000, while running costs would range between R 2 400 000 and R 6 700 000 per annum, depending on the number and size of fish to be released.

7.7. Conclusion

Further work is required on the dynamics of wild *A. japonicus*, particularly establishing whether the stock is in fact recruit limited or not, and also into whether the compliance with traditional management can be improved prior to the implementation of stock enhancement. However, within the scope of this study, there is no evidence to suggest that stock enhancement should not be further investigated as a management tool for *A. japonicus* in South Africa. Further research is required with regard to many aspects of stocking, however, in most cases the ability exists to acquire the necessary information and the technology exists to implement the necessary precautions. The ability to produce large numbers of juvenile *A. japonicus* in captivity in a potentially economically feasible manner and the technology to achieve an environmentally safe stock enhancement programme do exist.

Provided stock enhancement is found to be a suitable management option in terms of the genetic and ecological studies that need to be conducted, it cannot be used effectively without being integrated into a broader management plan. Stock enhancement on its own cannot achieve the goals of facilitating the recovery of *A. japonicus* stocks in South Africa. It will, therefore, need to be integrated into a broader management plan, being used in conjunction with traditional management tools.

With strict guidelines and the correct management based on a sound scientific approach, stock enhancement may indeed be the tool that managers have been looking for to use in conjunction with traditional to facilitate the recovery of stock enhancement of *A. japonicus* in South Africa.

REFERENCES

AADLAND, D. and A. CAPLAN 2003 – Willingness to pay for curbside recycling with detection and mitigation of hypothetical bias. *American Journal of Agricultural Economics* **85**(2): 492-502.

ANON. 1997 – Sport fishery use and value on lower Snake River reservoirs – Summary of phase 1 studies. US Army Corps of Engineers, Walla Walla district.

ANON. 2004 – Recreational Fishing Information Brochure, December 2004. *Department of environmental affaires and tourism.* Rogge Bay, Cape Town. www.mcm-deat.gov.za/recreational/bag_limits.html last accessed 18 April 2007.

ANON. 2005 – Recreational fishing information brochure, December 2005. Department of Environmental Affairs and Tourism, Republic of South Africa.

ANON. 2005 – *White Paper. A marine fisheries policy for South Africa*. Cape Town; department of environmental affairs and tourism

AQUACULTURE SA, 2003 – Mulloway aquaculture in South Australia. Primary industries and resources SA – Fact sheet. www.pir.sa.gov.au/bytserve/aquaculture/species_profiles/mulloway_fs.pdf last accessed 18 April 2007.

ARROW, K.J., SOLOW, R., LEAMER, E., RADNER, R. and H. SCHUMAN 1993 – Report of the NOAA Panel on contingent valuation. *National Oceanic and Atmospheric Administration Federal Register* **58**(10).

ATTWOOD, C.G. and B.A. BENNETT 1995 – A procedure for setting daily bag limits on the recreational shore-fishery of the south-western cape, South Africa. *South African Journal of Marine Science* **15**: 241-251.

BARKER, G. 2000 – Novel methods to reduce disease in aquaculture. *Fish Veterinary Journal* **5**: 66-71.

BARTLEY, D.M., BONDAD-REANTASO, M.G. and R.P. SUBASINGHE 2006 – A risk analysis framework for animal health management in marine stock enhancement programs. *Fisheries Research* **80**: 28-36.

BATEMAN, I.J., COLE, M., COOPER, P., GEORGIOU, S., HADLEY, D. and G.L. POE 2004 – On visible choice sets and scope sensitivity. *Journal of Environmental Economics and Management* **47**: 71-93.

BATTAGLENE, S.C. and R.W. TALBOT 1994 – Hormone induction and larval rearing of Mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae). *Aquaculture* **126**: 73-81.

BELL, J.D. 2004 – Management of restocking and stock enhancement programs: The need for different approaches. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand. (Eds). Oxford: Blackwell Publishing Ltd: 213-224.

BELL, J.D., BARTLEY, D.M., LORENZEN, K. and N.R. LONERAGAN 2006 – Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. *Fisheries Research* **80**: 1–8.

BENNETT, R. and R. TRANTER 1998 – The dilemma concerning choice of contingent valuation willingness to pay elicitation format. *Journal of Environmental Planning and Management* **41**(2): 253-258.

BERGMAN, P.K., JEFFERTS, K.B., FISCUS, H.F. and R.L. HAGER 1968 – A preliminary evaluation of an implanted coded wire fish tag. *Washington Department of Fisheries, Fisheries Research Paper* **3**: 63-84.

BERT, T.M., MCMICHAEL, JR, R.H., CODY, R., FORSTCHEN, A.B., HALSTEAD, W.G., O'HOP, J., RANSIER,J.M., TRINGALI, M.D., WINNER, B.L., KENNEDY, F.S., LEBER, K.M. and C.L. NEIDIG 2003 – Evaluating stock enhancement strategies: a multi-disciplinary approach. *UJNR Technical Report* **30**: 105-126

BLANKENSHIP, H.L. 1990 – Effects of time and fish size on coded wire tag loss from chinook and coho salmon. *American Fisheries Society Symposium* **7**: 237-243.

BLANKENSHIP, H.L. and K.M. LEBER 1995 – A responsible approach to marine stock enhancement. In *Uses and effects of cultured fishes in aquatic ecosystems.* Schramm Jr., H.L. and R.G. Piper (Eds.) *American fisheries symposium* **15**: 167-175.

BLANKO, G., CAGIGAS, E., VAZQUEZ, E. and J.A. SANCHEZ. 1998 – Genetic impact of introduced domesticated strains of brown trout, *Salmo trutta*, on native Spanish populations. In *Stocking and introduction of fish.* Cowx, I.G. (Ed). Oxford: Fishing News Books: 371-379.

BLAXTER, J.H.S. 1975 – Reared and wild fish – how do they compare? In *10th European Symposium on Marine Biology, Ostend, Belgium, September 1975 Vol 1. Mariculture.* Persoone, G. and E. Jaspers (Eds). Universal press, Belgium: 11-26.

BLOOMER, P. 2004 – Feasibility study into the application of genetic techniques for determining fish stock identity of transboundary populations in the BCLME region. Project LMR/CF/03/04.

BOHM, P. 1972 – Estimating demand for public goods: An experiment. *Scandinavian Journal of Economics* **3**: 113-130.

BOHM, P. 1979 – Estimating willingness-to-pay: Why and how? *Scandinavian Journal of Economics* **81**: 142-153.

BOTELHO, A. and L. PINTO 2002 – Hypothetical, real and predicted real willingness to pay in open-ended surveys: experimental results. *Applied Economics Letters* **9**: 993-996.

BRINK, D. 2003 – Overview of aquaculture in South Africa: 2003. Division of Aquaculture, University of Stellenbosch.10pp.

BROTHERS, E.B. 1990 – Otolith marking. *American Fisheries Society Symposium* **7**: 183-202.

BROUWER, S.L., MANN, B.Q., LAMBERTH, S.J., SAUER, W.H.H. and C. ERASMUS 1997 – A survey of the South African Shore-angling fishery. *South African Journal of Marine Science* **18**: 165-177.

BROWN, T.C., AJZEN, I. and D. HRUBES 2003 – Further tests of entreaties to avoid hypothetical bias in referendum contingent valuation. *Journal of Environmental Economics and Management* **46**: 353–361.

BUCHMANN, K., LARSEN, J.L. and B. THERKILDSEN 2001 – Improved re-capture rate of sea-ranched vaccinated Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* **24**: 245-248.

BUCKLEY, R.M. and H.L. BLANKENSHIP 1990 – Internal extrinsic identification systems: Overview of implanted wire tags, otolith marks, and parasites. *American Fisheries Society Symposium* **7**: 173-182.

BURG, T.M., TRITES, A.W. and M.J. SMITH 1999 – Mitochondrial and microsatellite DNA analyses of harbour seal population structure in the northeast Pacific Ocean. *Canadian Journal of Zoology* **77**: 930–943.

CANTRELL, R.N., GARCIA, M., LEUNG, P. and D. ZIEMANN 2004 – Recreational anglers' willing to pay for increased catch rates on Pacific threadfin (*Polydactylus sexfilis*) in Hawaii. *Fisheries research* **68**: 149-158.

CARRILLO, J., KOUMOUNDOUROS, G., DIVANACH, P. and J. MARTINEZ 2001 – Morphological malformations of the lateral line in gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture* **192**: 281-290.

CARSON, R. 1997 – Contingent valuation: Theoretical advances and empirical tests since the NOAA panel. *American Journal of Agricultural Economics* **79**(5): 1501-1508.

CARSON, R.T, FLORES, N.E. and N.F. MEADE 2000 – Contingent valuation: Controversies and evidence. *Forthcoming Environmental and Resource Economic*s. July 2000.

CARSON R. T., HANEMANN, W.M., KOPP, R.J., KROSNICK, J.A., MITCHELL, R.C., PRESSER, S., RUUD, P.A., SMITH, V.K., CONAWAY, M. and K. MARTIN 1996 – Was the NOAA panel correct about contingent valuation? Discussion Paper 96-20.

CARSON, R. and R. MITCHELL 1993 – The issue of scope in contingent valuation studies. *American Journal of Agricultural Economics* **75**(5): 1263-1268.

CARSON, R., MITCHELL, R., HANEMANN, M., KOPP, R., PRESSER, S. and P. RUUD 2003 – Contingent valuation and lost passive use damages from the Exxon Valdez oil spill. *Environmental and Resource Economics* **25**: 257-286.

CARVALHO, G.R. and T.F. CROSS 1998 – Genetic considerations in stocking Atlantic salmon, *Salmo salar*. In *Stocking and introduction of fish.* Cowx, I.G. (Ed). Oxford: Fishing News Books: 355-370.

CATALANO, M.J., CHIPPS, S.R., BOUCHARD, M.A. and D.H. WAHL 2001 - Evaluation of injectable fluorescent tags for marking centrarchid fishes: Retention rate and effects on vulnerability to predation. *North American Journal of Fisheries Management* **21**: 911–917.

CHAMBERS, C. and P. CHAMBERS 1998 – Contingent valuation of quasi-public goods: validity, reliability and application to valuing a historic site. *Public Finance Review* **26**(2): 137-155.

CHAMBERS, G.K. and E.S. MACAVOY. 1999 – Molecular genetic analysis of Hybridization. *Wellington, New Zealand: Dept. of Conservation,* 1999.

CHAMP, P. and R. BISHOP 2001 – Donation payment mechanisms and contingent valuation: an empirical study of hypothetical bias. *Environmental and Resource Economics* **19**: 383-402.

COWLEY, P.D., KERWATH, S., NAESJE, T.F., CHILDS, A.R., GILLESPIE, L.D., LEGGITT, C., THORSTAD, E.B. and F. OKLAND 2006 – Space use patterns and movement of juvenile dusky kob *Argyrosomus japonicus* in the Great Fish Estuary (South Africa): implications for management. *NINA Report* **119**. 32 pp.

COWX, I.G. 1998 – Stocking strategies: issues and opportunities for future enhancement programs. In *Stocking and introduction of fish*. Cowx. I.G. (Ed.). Oxford; Fishing News Books: 3-13.

CROSS, T.F. 1999 – Genetic considerations in enhancement and ranching of marine and anadromous species. In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford: Fishing News Books: 37-48.

CUMMINGS, R. and L. TAYLOR 1999 – Unbiased value estimates for environmental goods: A cheap talk design for the contingent valuation method. *American Economic Review* **89**(3): 649-666.

DARWIN, C. 1872 – The origin of species. London: John Murray: pp 458.

DAVENPORT. J., EKARATNE. S.U.K., WALGAMA, S.A., LEE, D. and J.M. HILLS 1999 – Successful stock enhancement of a lagoon prawn fishery at Rekawa, Sri Lanka using cultured post-larvae of penaeid shrimp. *Aquaculture* **180**: 65-78.

DEAT 2002 – Ecological risk assessment, integrated environmental management, Series 6, Department of Environmental Affairs and Tourism, Pretoria.

DEAT 2002 – Screening, information series 1, Department of Environmental Affairs and Tourism (DEAT), Pretoria. www.mcm-deat.gov.za last accessed 11 April 2007

DEAT 2006 – Draft Guidelines for marine ranching in South Africa. Department of Environmental Affairs and Tourism. www.mcm-deat.gov.za last accessed 11 April 2007

Department of Environmental Affairs and Tourism Chief Directorate: Marine and Coastal Management. 2005 – Media statement on the issuing of a draft traditional line fish policy for notice and comment and public inquiry. www.mcm-deat.gov.za last accessed 11 April 2007

DEWEY, M.R. and S.J. ZIGLER 1996 – An evaluation of fluorescent elastomer for marking bluegill sunfish in experimental studies. *The Progressive Fish Culturist* **58**: 219-220.

DODD, Q. 2003 – Florida hatchery works to increase red drum stocks in Tampa Bay. *Hatchery International 2003.*

DOUPÉ, R.G., PARTRIDGE, G.J. and A.J. LYMBERY 2003 – Visible implant fluorescent elastomer tags as pedigree markers for applied aquaculture: an evaluation using black bream *Acanthopagrus butcheri*. *Aquaculture Research* **34**: 681-683.

DRAWBRIDGE, M. A. 2002 – The role of aquaculture in the restoration of coastal fisheries. In: *Ecological Aquaculture, The Evolution of the Blue Revolution*. Costa-Pierce, B. A. (Ed.). Blackwell Science, Oxford: 314–336.

DUNCOMBE, W. 1996 – Public expenditure research: what have we learned? *Public Budgeting and Finance* **16**(2): 26-59.

EGLI, D.P. and R.C. BABCOCK 2004 – Ultrasonic tracking reveals multiple behavioural modes of snapper (*Pagus auratus*) in a temperate no-take marine reserve. *ICES Journal of Marine Science* **61**: 1137-1143.

FAIRCHILD, E.A. and W.H. HOWELL 2004 – Factors affecting the post-release survival of cultured juvenile *Pseudopleuronectes americanus. Journal of Fish Biology* **65**: 69-87.

FAO 2005 – Fishery country profile. The Republic of South Africa. http://www.fao.org/fi/fcp/en/ZAF/profile.htm last accessed: 11 April 2007

FERNANDEZ, C., LEON, C.J., STEEL, M.F.J. and F.J. VAZQUEZ-POLO 2004 – Bayesian analysis of interval data contingent valuation models and pricing policies. *Journal of Business and Economic Statistics*. October 2004.

FISHERIES AND MARINE ENVIRONMENTAL RESEARCH FACILITY 2003 – Mulloway stock enhancement. www.famer.unsw.edu.au/mulloway/home.htm last assessed 18 April 2007.

FRIEDLANDER, A.M. and D.A. ZIEMANN 2003 – Impact of hatchery releases on the recreational fishery for Pacific threadfin (*Polydactylus sexfilis*) in Hawaii. *Fisheries Bulletin* **101**: 32-43.

FRYKBLOM, P. 1997 – Hypothetical question modes and real willingness to pay. *Journal of Environmental Economics and Management* **34**: 275-287.

FUJII, T. and M. NISHIDA 1997 – Comparison of the genetic variability between wild and artificially raised Japanese flounder (*Paralichthys olivaceus*). *Bulletin of the National Institute of Aquaculture. Supplement* **3**: 73.

FUSHIMI, H. 2001 – Production of juvenile marine finfish for stock enhancement in Japan. *Aquaculture* **200**: 33-53.

GOLD, J.R. 2004 – Stock structure and effective size of red drum (*Sciaenops occelatus*) in the Northern Gulf of Mexico and implications relative to stock enhancement and recruitment. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand. (Eds). Oxford: Blackwell Publishing Ltd: 353-370.

GRIFFITHS, M.H. 1996 – Life history of the dusky kob *Argyrosomus japonicus* (Sciaenidae) off the east coast of South Africa. *South African Journal of Marine Science* **17**: 135-154.

GRIFFITHS, M.H. 1997a – Management of South African dusky kob *Argyrosomus japonicus* (Sciaenidae) based on per recruit models. *South African Journal of Marine Science* **18**: 213-228.

GRIFFITHS, M.H. 1997b – Influence of prey availability on the distribution of dusky kob *Argyrosomus japonicus* (Sciaenidae) in the great fish river estuary, with notes on the diet of early juveniles from three other estuarine systems. *South African Journal of Marine Science* **18**: 137-145.

GRIFFITHS, M.H. 1998 – Focus on the dusky kob. *Tagging News* **11.**

GRIFFITHS, M.H. and T. HECHT 1993 – Two South African *Argyrosomus hololepidotus* species: implications for management. In *Fish, Fishers and Fisheries. Proceedings of the Second South African Marine Linefish Symposium, Durban, October 1992.* Beckley, L.E. and R.P. van der Elst (Eds). *Special Publication Oceanographic Research Institute of South Africa* **2**: 19-22.

GRIFFITHS, M.H. and T. HECHT 1995 – Age and growth of South African dusky kob *Argyrosomus japonicus* (Sciaenidae) based on otoliths. *South African Journal of Marine Science* **16**: 119-128.

GRIFFITHS, M.H., ATTWOOD, C. and R. THOMAS 2000 – New management protocol for the South African linefishery.

GODFREY, B.P. 2003 – The potential of abalone stock enhancement in the Eastern Cape Province of South Africa. MSc thesis, Rhodes University, Grahamstown.

HAINES, B.G. and T. MODDE 1996 – Evaluation of marking techniques to estimate population size and first year survival of Colorado squawfish. North *American Journal of Fisheries Management* **16**: 905-912.

HAUSER, L., CARVALHO, G.R. and T.J. PITCHER 1998 – Genetic similarity of a phenotypically divergent population of the clupeid *Limnothrissa miodon* following its introduction into a man-made lake. In *Stocking and introduction of fish.* Cowx, I.G. (Ed). Oxford: Fishing News Books: 338-354.

HEDGECOCK, D. 1994 – Does variance in reproductive success limit population size of marine organisms: In *Genetics and evolution of aquatic organisms.* Beaumont, A.R. (Ed). New York: Chapman and Hall: 122-134.

HEYDORN, A.E.F., BANG, N.D. and A.F. PEARCE 1978 – Ecology of the Agulhas Current region: an assessment of biological responses to environmental parameters in the south-west Indian Ocean. *Transactions of the Royal Society of South Africa* **43**: 151-190.

HILBORN, R. 2004 – Population management in stock enhancement and sea ranching. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford; Blackwell Publishing Ltd: 201-209.

HOSSAIN, M.A.R., TANAKA, M. and R. MASUDA 2002 – Predator-prey interactions between hatchery-reared Japanese flounder juvenile, *Paralichthys olivaceus*, and sandy shore crab, *Matuta lunaris*: daily rhythms, anti-predator conditioning and starvation. *Journal of Experimental Marine Biology and Ecology* **267**: 1-14

HOWARTH, W. and C. LERIA 1999 – Legal issues relating to stock enhancement and marine ranching. In *Stock enhancement and sea ranching*. Howell, B.R., Morksness, E. and T. Svesand (Eds). Oxford; Fishing News Books: 509-525.

HOWELL, B. 1998 – Marine fisheries. In *Stocking and introduction of fish*. Cowx I.G. (Ed.) Oxford; Fishing News Books: 289-293.

HUNTINGFORD, F.A. 2004 - Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* **65**(Supplement A): 122-142.

IHSSEN, P.E., BOOKE, H.E., CASSELMAN, J.M., MCGLADE, J.M., PAYNE, N.R. and F.M. UTTER 1981 – Stock identification: materials and methods. *Canadian Journal of Fisheries and Aquatic Science* **38**: 1838–1855.

JEFFERTS, K.B., BERGMAN. P.K. and H. FISCUS 1963 – A coded wire identification system for Macro-organisms. *Nature* **198**(4879): 460-462.

JØRGENSEN, H.B.H., HANSEN, M.M., BEKKEVOLD, D., RUZZANTE, D.E. and V. LOESCHCKE 2005 – Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Molecular Ecology* **10**: 1111-1127.

JORSTAD, K.E., SKAALA, O. and G. NÆVDAL 1999 – Genetic diversity and the Norwegian sea ranching program: an evaluation. In *Stock enhancement and sea ranching*. Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford: Fishing News Books: 49-62.

KAERIYAMA, M. and R.R. EDPALINA 2004 – Evaluation of the biological interactions between wild and hatchery populations for sustainable fisheries management of Pacific salmon. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford; Blackwell Publishing Ltd: 247-259.

KAHNEMAN, D. and J. KNETSCH 1992 – Valuing public goods: The purchase of moral satisfaction. *Journal of Environmental Economics and Management* **22**: 57-70.

KELLISON, G.T., EGGLESTON, D.B. and J.S. BURKE 2000 – Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dendatus*). *Canandian Journal of Fisheries and Aquatic Science* **57**: 1870-1877.

KENYON, W. and G. EDWARDS-JONES 1998 – What level of information enables the public to act like experts when evaluating ecological goods? *Journal of Environmental Planning and Management* **41**: 463-476.

KITADA, S. 1999 – Effectiveness of Japan's stock enhancement programmes: current perspectives. In *Stock enhancement and sea ranching.* Howell, B.R., Morksness, E. and T. Svesand (Eds). Oxford; Fishing News Books: 103-131.

KITADA, S. and H. KISHINO 2004 – Examining genetic effect hypotheses of hatchery fish on wild populations: a Bayesian approach. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T.Svesand. (Eds). Oxford; Blackwell Publishing Ltd. 153-167.

KLOPPER, A.W. 2005 - Intraspecific genetic variation in the percoid teleosts *Argyrosomus japonicus* (Temminck & Schlegel, 1843) and *Pomadasys commersonnii* (Lacepède, 1801), as inferred from the mitochondrial control region. MSc thesis, University of Pretoria, Pretoria.

KNIGHT, R.L. and S.F. BATES 1995 – A new Century for Natural Resources Management. Island Press: Washington DC 20009.

KREIBERG, H. 2000 – Stress and anaesthesia. In *The Laboratory Fish.* Ostrander. G.K. (Ed.). London: Academic Press: 503-511.

KUWADA, H., MASUDA, R., SHIOZAWA, S., KOGANE, T., IMAIZUMI, K. and K. TSUKAMOTO 2000 – Effect of fish size, handling stresses and training procedure on the swimming behaviour of hatchery-reared striped jack: implications for stock enhancement. *Aquaculture* **185**: 245-256.

LAM, T.J. – Genetics & Biotechnology in Aquaculture: Status, Promises & Issues. www.aquachallenge.org/workshop_materials/tom_lam.pdf last accessed 18 April 2007.

LANGTON, R. and W. WILSON 1998 – Economic considerations for fish stocking and enhancement. In *Stocking and Introduction of Fish.* Cowx, I.G. (Ed). Oxford; Fishing News Books: 14-21.

LAUFLE, J.C., JOHNSON, L. and C.L. MONK 1990 – Tattoo-ink marking method for batch identification of fish. *American Fisheries Society Symposium* **7**: 38-41.

LEBER, K., KITADA, S., BLANKENSHIP, L. and T. SVASAND. 2004 - Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition. Oxford: Blackwell Publishing Ltd: 562pp.

LEBER, K.M. 1999 – Rationale for an experimental approach to stock enhancement. In . In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford; Fishing News Books: 63-74.

LEBER, K.M. 2002 – Advances in marine stock enhancement: Shifting emphasis to theory and accountability. In *Responsible marine aquaculture.* Stickney, R.R. and McVey, J.P. (Eds). CAB International: 79-90.

LEBER, K.M. 2004 – Marine stock enhancement in the USA: Status, trends and needs. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand. (Eds). Oxford; Blackwell Publishing Ltd: 11-24.

LEE. C.S. and A.C. OSTROWSKI 2001 – Current status of marine finfish larviculture in the United States. *Aquaculture* **200**: 89-109.

LI, Q., PARK, C., ENDO, T. and A. RIJIMA. 2004 - Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). *Aquaculture* **235**: 207-222.

LIAO, I.C., SU, M.S. and E.M. LAEÑO 2003 – Status of research in stock enhancement and sea ranching. *Reviews in Fish Biology and Fisheries* **13**: 151-163.

LIST, J. and C. GALLET 2001 – What experimental protocols influence disparities between actual and hypothetical stated values? *Environmental and Resource Economics* **20**: 241-254.

LIST, J. and J. SHOGREN 1998 – Calibration of the difference between actual and hypothetical valuation in a field experiment. *Journal of Economic Behaviour and Organization* **37**: 193-205.

LIU, M. 2004 – Possible procedures for estimating the environmental carrying capacity of coastal water bodies with respect to priority contaminants – Nutrients and heavy metals. *"Reversing Environmental Degradation Trends in the South China Sea and Gulf of Thailand"* Fifth Meeting of the Regional Working Group for the Land-Based Pollution Component of the UNEP/GEF Project: 1-9.

LUO, J., HARTMAN K.J., BRANDT S.B., CERCO C.F. and T.H. RIPPETOE 2001 – A spatially-explicit approach for estimating carrying capacity: An application for the Atlantic menhaden (Brevoortia tyrannus) in Chesapeake Bay. *Estuaries* **24** (4): 545-556.

MAHNKEN, C.V.W., FAIRGRIEVE, W.T., WAKNITZ, F.W., MAYNARD, D.J. and H.L. BLANKENSHIP 2004 – Lessons for marine fish enhancement: Experiences with Pacific salmon. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 91-105.

MASUDA, R. 2004 – Behavioural approaches to fish stock enhancement: A practical review. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 83-90.

MATSUOKA, M. 1987 – Development of the skeletal tissues in red sea bream. *Bulletin of Seikai Regional Fisheries Research Laboratory* **65**: 1-112.

MAYNARD, D.J., LARAE, A.L., MCDOWELL, FLAGG, T.A. and C.V.W. MAHNKEN 2001 – Effect of predator avoidance training on postrelease survival of fall chinook salmon. In *Development of a natural rearing system to improve supplemental fish quality. 1996-1998 Progress report.* Maynard, D.J., Berejikian, B.A., Flagg, T.A. and C.V.W. Mahnken (Eds). Project No. 199105500. 174 pp.

MCEACHRON, L.W., COLURA, R.L., BUMGUARDNER, B.W. and R. WARD 1998 – Survival of stocked red drum in Texas. *Bulletin of Marine Science* **62**(2): 359-368.

MCFARLAND, W.N. 1959 – A study of the effects of anaesthetic on behaviour and physiology of fishes. *Publication of the Institute of Marine Science, University of Texas* **6**: 23-55.

MCFARLANE, G.A., WYDOSKI, R.S. and E.D. PRINCE 1990 – Historical review of the development of external tags and marks. *American Fisheries Society Symposium* **7**: 9-29.

MEINERTZ, J.R., GAIKOWSKI, M.P., STEHLY, G.R., GINGERICH, W.H, and J.A. EVERED 2001 - Oxytetracycline depletion from skin-on fillet tissue of coho salmon fed oxytetracycline medicated feed in freshwater at temperatures less than 9°C. *Aquaculture* **198**: 29-39.

MIRANDA, C.D. and R. ZEMELMAN 2002 – Bacterial resistance to oxytetracycline in Chilean salmon farming. *Aquaculture* **220**: 31-47.

MOLONY, B.W., LENANTON, R., JACKSON, G. and J. NORRISS 2003 – Stock enhancement as a management tool. *Reviews in Fish Biology and Fisheries* **13**: 409-432.

MOORE, D. and J. SEEB – Alaska department of fish and game, Finfish genetic policy. www.cf.adfg.state.ak.us/geninfo/research/genetics/publish/posters/policy.pdf last accessed 18 April 2007.

MORI, M. and K. HIGUCHI 2004 – Genetic monitoring system for SAM strains utilizing DNA markers. *International Congress Series* **1260**: 187– 190.

MURPHY, J.J. and T.H. STEVENS 2004 - Contingent Valuation, Hypothetical Bias and Experimental Economics. Prepared for the NAREA-CAES Conference June 20-23, 2004 Halifax, Nova Scotia.

MURPHY, J.J., ALLEN, P.G., STEVENS, T.H. and D. WEATHERHEAD 2005 – A meta-analysis of hypothetical bias in stated preference valuation. *Environmental and Resource Economics* **30**: 313-325.

MUSHIAKE, K. and K. MUROGA 2004 – Fish health management in seed production. In *Stock enhancement and sea ranching. Developments, pitfalls and* *opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 119-129.

MUSTAFA, S. 2003 – Stock enhancement and sea ranching: objectives and potential. *Reviews in Fish Biology and Fisheries* **13**: 141-149.

MUSTAFA, S., SAAD, S. and R.A. RAHMAN 2003 – Species studies in sea ranching: an overview and economic perspectives. *Reviews in Fish Biology and Fisheries* **13**: 165-175.

NASH, C.E., P.R. BURBRIDGE, and J.K. VOLKMAN (editors). 2005 – Guidelines for ecological risk assessment of marine fish aquaculture. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-71, 90 p.

NIELSON, B.R. 1990 – Twelve-year overview of fluorescent grit marking of cutthroat trout in bear lake, Utah-Idaho. *American Fisheries Society Symposium* **7**: 42-46.

NODTVEDT, M., FERNO, A., GJOSÆTER, J. and P. STEINGRUND 1999 – Anti-predator behaviour of hatchery reared and wild juvenile atlantic cod (*Gadus morhua* L.) and the effect of predator training. In *Stock enhancement and sea ranching.* Howell, B.R., Morksness, E. and T. Svesand (Eds). Oxford; Fishing News Books: 350-362.

NORTHWEST MARINE TECHNOLOGY, INC. 2000 – Coded wire tag project manual guidelines on planning and conducting projects using CWT and associated equipment. www.nmt.us/products/cwt/cwt.htm last accessed 18 April 2007.

NORTHWEST MARINE TECHNOLOGY, INC. 2002 - Instructions for using the single-shot Coded Wire Tag injector.

NORTHWEST MARINE TECHNOLOGY, INC. 2004 – Tagging small fish with visible implant fluorescent elastomer. www.nmt.us/support/appnotes/ape03.pdf last accesses 18 April 2007.

NORTHWEST MARINE TECHNOLOGY, INC. 2005 – Manual elastomer injection systems: instructions for use. www.nmt.us/support/appnotes/ape06.pdf last accessed 18 April 2007.

NORTHWEST MARINE TECHNOLOGY, INC. 2006 – Product catalogue, January 1, 2006.

O'CONNELL, M. and J.M. WRIGHT 1997 – Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* **7**: 331-363.

OELLERMAN, L.K. 2000 – Biological information required for the proposed intensive, recirculating fish farm in Oranjemund, Namibia. Part 1: Dusky kob (*Argyrosomus japonicus*). *Unpublished.*

OLLA, B.L. and M.W. DAVIS 1989 – The role of learning and stress in predator avoidance of hatchery reared coho salmon (*Oncorhynchus kisutch*) juveniles. *Aquaculture* **76**: 209-214.

OLSEN, E.M and L.A. VOLLESTAD 2001 – An evaluation of visible implant elastomer for marking age-0 brown trout. *North American Journal of Fisheries Management* **21**: 967–970.

OTTERA, H. KRISTIANSEN, T.S., SVASAND, T., NODTEDT, M. and A. BORGE 1999 – Sea ranching of Atlantic cod (*Gadus morhua* L.): Effects of release strategy on survival. In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford; Fishing News Books: 293-305.

PEREZ-ENRIQUEZ, R., TAKAGI, M. and N. TANIGUCHI 1999 – Genetic variability and pedigree tracing of a hatchery-reared stock of red sea bream *Pagrus major* used for stock enhancement, based on microsatellite DNA markers. *Aquaculture* **173**: 413–423.

POLLARD, M.J., KINGSFORD, M.J. and S.C. BATTAGLENE 1998 – Chemical marking of juvenile snapper, *Pagrus auratus* (Sparidae), by incorporation of strontium into dorsal spines. *Fisheries Bulletin* **97**: 118-131.

PULATSU, S. 2003 - The Application of a Phosphorus Budget Model Estimating the Carrying Capacity of Kesikköprü Dam Lake. *Turkish Journal of Veterinary and Animal Sciences* **27**: 1127-1130.

RASMUSSEN, G. and P. GEERTZ-HANSEN 1998 – Stocking fish in Denmark. In *Stocking and introduction of fish.* Cowx, I.G. (Ed). Oxford; Fishing News Books: 14-21.

RIESENBICHLER, R., RUBEN, S., WETZEL, L. and S. PHELPS 2004 – Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss.* In *Stock enhancement and sea ranching. Developments,* *pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 371-383.

RIMMER, M.A. and D.J. RUSSELL 1998 – Survival of stocked barramundi, *Lates calcarifer* (bloch), in a coastal river system in far northern Queensland, Australia. *Bulletin of Marine Science* **62**(2): 325-335

SADLER, B. 1996 – Environmental assessments in a changing world: Evaluating practice to improve performance, final report of the international study of the effectiveness of environmental assessment, International association for impact assessment, Canadian Environmental Assessment Agency, Ottawa, Canada. In DEAT 2002 – Screening, information series 1, Department of Environmental Affairs and Tourism (DEAT), Pretoria.

SÆGROV, H. and O.T. SKILBREI 1999 – Can stocking programmes affect the predator stocks and decrease the survival of the wild Atlantic salmon juveniles? In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford; Fishing News Books: 182-188.

SAKAGAWA, G.T. 1995 – Assessment methodologies and fisheries management. In *Assessment methodologies and fisheries management. Proceedings of the World Fisheries Congress Theme 5.* Sakagawa, G.T. (Ed.) Science Publishers Inc., Lebanon, U.S.A.: 1-3.

SAMUELSEN, O.B. 1989 - Degradation of Oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of Oxytetracycline in the sediment from a fish farm. *Aquaculture* **83**: 7-16.

SANCHEZ-LAMADRID, A. 2004 – Effectiveness of releasing gilthead sea bream (*Sparus aurata,* L.) for stock enhancement in the bay of Cádiz. *Aquaculture* **231**: 135-148.

SAUER, W.H.H., PENNEY, A.J.A., ERASMAS, C., MANN, B.Q., BROUWER, S.L., LAMBERTH, S.J. and T.J. STEWART 1997 – An evaluation of attitudes and responses to monitoring and management measures for the South African boat-based linefishery. *South African Journal of Marine Science* **18**: 147-163.

SAUER, W.H.H., HECHT, T., BRITZ, P.J. and D. MATHER 2003a – An economic and sectoral study of the South African fishing industry. Volume 1: Economic and regulatory principles, survey results, transformation and socio-economic impact. Report prepared for Marine and Coastal Management by Rhodes University.

SAUER, W.H.H., HECHT, T., BRITZ, P.J. and D. MATHER 2003b – An economic and sectoral study of the South African fishing industry. Volume 2: Fishery Profile. Report prepared for Marine and Coastal Management by Rhodes University.

SEEB, J.E., HABICHT, C., OLSEN, J.B., BENTZEN, P. and L.W. SEEB 1998 – Allozyme, mtDNA, and microsatellite variants describe structure of populations of pink and sockeye salmon in Alaska.

SEKINO, M., HARA, M. and N. TANIGUCHI 2002 – Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus*. *Aquaculture* **213**: 101-122.

SEKINO, M., SAITOHA, K., YAMADAB, T., KUMAGAIB, A., HARAC, M. and Y. YAMASHITA 2003 – Microsatellite-based pedigree tracing in a Japanese flounder *Paralichthys olivaceus* hatchery strain: implications for hatchery management related to stock enhancement program. *Aquaculture* **221**: 255–263.

SERAFY, J.E., AULT, J.S., CAPO, T.R. and D.R. SCHULTZ 1999 – Red drum, *Sciaenops ocellatus* L., stock enhancement in the Biscayne Bay, FL, USA: assessment of releasing unmarked early juveniles. *Aquaculture Research* **30**: 737-750.

SNOWBALL, J.D. 2000 – Towards more accurate measurement of the value of the arts to society: Economic impact and willingness to pay studies at the Standard Bank National Arts Festival. Unpublished Masters Thesis: Department of Economics, Rhodes University, Grahamstown, South Africa.

SNOWBALL, J.D. 2005 - The economic valuation of cultural events in developing countries: combining market and non-market valuation techniques at the South African National Arts Festival. PhD Thesis: Department of Economics, Rhodes University, Grahamstown, South Africa.

SOLOMON, D.J. 2005 – Coded wire tag project manual: Guidelines on use of coded wire tags and associated equipment. Northwest Marine Technology Inc. www.mnt.us

SPANGGAARD, B., JORGENSEN, F., GRAM, L. and H.H. HUSS 1993 - Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. *Aquaculture* **115**: 195-207.

SUMMERFELT, R.C. and L.S. SMITH 1990 – Anaesthesia, surgery and related techniques. In *Methods for fish biology*. Schreck, C.B. and P.B. Moyle (Eds). American Fisheries Society, Bethesda, Maryland: 213-272.

SVÅSAND, T. 2004 – Why juvenile quality and release strategies are important factors for success in stock enhancement and sea ranching. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 61-70.

TABATA, K., KISHIOKA, H., TAKAGI, M., MIZUTA, A. and N. TANIGUCHI 1997 – Genetic diversity of five strains of red sea bream *Pagrus major* by RFLP 85.

TANIGUCHI, N. 2003 – Genetic factors in broodstock management for seed production. *Reviews in Fish Biology and Fisheries* **13**: 177-185.

TANIGUCHI, N. 2004 – Broodstock management for stock enhancement programs of marine fish with assistance of DNA marker (a review). In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand (Eds). Oxford: Blackwell Publishing Ltd: 329-338.

TANIGUCHI, N., SUMANTADINATA, K. and S. IYAMA 1983 – Genetic change in the first and second generations of hatchery reared black sea bream. *Aquaculture* **35**: 309-320.

TAYLOR, M.D., FIELDER, D.S. and I.M. SUTHERS 2005a – Batch marking of otoliths and fin spines to assess the stock enhancement of *Argyrosomus japonicus*. *Journal of Fish Biology* **66**: 1149-1162.

TAYLOR, M.D., PALMER, P.J., FIELDER, D.S. and I.M. SUTHERS 2005b – Responsible estuarine finfish stock enhancement: an Australian perspective. *Journal of Fish Biology* **67**(2): 299-331.

THOMPSON, B., THROSBY, D. and G. WITHERS 1983 – Measuring community benefits from the arts. Research paper 261, School of Economic and Financial Studies: Macquarie University.

THROSBY, D. and G. WITHERS 1986 – Strategic bias and demand for public goods: theory and application to the arts. In *Cultural economics: The arts, the heritage and the media industries. Volume 2.* Towse, R. (Ed.). 1997. Edward Elgar: Cheltenham.

TRANAH, G.J., KINCAID, H.J., KRUEGER, C.C., CAMPTON, D.E. and M. BERNIE 2001 – Reproductive Isolation in Sympatric Populations of Pallid and Shovelnose Sturgeon. *North American Journal of Fisheries Management* **21**: 367–373.

TSUKAMOTO, K., KUWADA, H., UCHIDA, K., MUSADA, R. and Y. SAKUKARA 1999 – Fish quality and stocking effectiveness: Behavioural approach. In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford: Fishing News Books. A division of Blackwell Science Ltd: 205-218.

VAN DER ELST, R.P., RADEBE, P.V. and A. GOVENDER 2000 – Status of the linefishery: Pomatomidae. *South African marine linefish status reports* **2000**: 74-67.

VAN ROOYEN, P., MARKOVINA, M. and R. BENNETT 2004 – Finfish Africa: Production of hatchery reared dusky kob *Argyrosomus japonicus* fingerlings. *Unpublished business plan*.

VAN ROOYEN, P., MARKOVINA, M., BENNETT, R. and R. PALMER 2005 – A preliminary report on the feasibility of restocking and culture of dusky kob *Argyrosomus japonicus* in the Plettenberg Bay area. *Unpublished report*: iii + 53pp.

VREELAND, R.R. 1990 – Random-sampling design to estimate hatchery contributions to fisheries. *American Fisheries Society Symposium* **7**: 691-707.

WAPLES, R.S. and J. DRAKE 2004 – Risk/benefit considerations for marine stock enhancement: a pacific salmon perspective. In *Stock enhancement and sea ranching. Developments, pitfalls and opportunities. Second edition.* Leber, K.M., Kitada, S., Blankenship, H.L. and T. Svasand. (Eds). Oxford; Blackwell Publishing Ltd: 260-306.

WARD, R.D. 2000 – Genetics in fisheries management. *Hydroglobia* **420**: 213-232.

WEDGEWOOD, A. and K. SANSOM 2003 – Willingness-to-pay surveys – A streamlined approach – Guidance notes for small town water services. Water, Engineering and Development Centre, Loughborough University, UK: xiv + 234 pp.

WESTERS, H. 1986 – Engineering advances related to new production capabilities. In *Fish culture in fisheries management.* Stroud, R.H. (Ed.). Fish Culture Section and Fisheries Management Section of the American Fisheries Society, Bethesda, Maryland: 229-233.

WHITFIELD, A.K. 1998 – Biology and ecology of fishes in Southern African estuaries. *Ichthyological monographs of the J.L.B. Smith Institute of Ichthyology,* No. 2, 223pp.

WILLIS, T.J. and R.C. BABCOCK 1998 – Retention and *in situ* detectability of visible implant fluorescent elastomer (VIFE) tags in *Pagrus auratus* (Sparidae). *New Zealand Journal of Marine and Freshwater Research* **32**: 247-254.

WOODWARD, A.G. 2000 – Red drum stock enhancement in Georgia: A responsible approach. *Atlantic Coast Red Drum Assessment Group*, April 2000.

YAMASHITA, Y. and H. YAMADA 1999 – Release strategy for Japanese flounder fry in stock enhancement programs. In *Stock enhancement and sea ranching.* Howell, B.R., Moksness, E. and T. Svasand (Eds). Oxford: Fishing News Books. A division of Blackwell Science Ltd: 191-204.

YE, Y., LONERAGAN, N., DIE, D., WATSON, R. and B. HARCH 2005 – Bioeconomic modelling and risk assessment of the tiger prawn (*Penaeus esculentus*) stock enhancement in Exmouth Gulf, Australia. *Fisheries Research* **73**: 231-249.

APPENDIX 1

Plettenberg Bay Angler Willingness to pay Survey – Beach survey

Date: ________________ Location:_______________

Where are you from? ______________________________

Do you have a recreational fishing permit? □ Yes □ No

A1. Where do you mainly fish? (Can tick more than one)

□ Estuary □ surf □ rock □ deep sea

2. How many fishing trips do you go on in a year? _____________

3. How much do you spend per year on fishing equipment such as rods, reels, bait etc. (not including vehicles or boats)? R __________

B1. Do you target kob?

□ yes □ no □ refused to answer

2. How many kob do you catch per year? _____________

3. How often do you do you reach your bag limit for kob?

□ never □ hardly ever □ sometimes □ often □ all the time

4. What size range do you catch most often?

□ 0-40cm □ 40-60cm □ 60-100cm □ >100cm

5. Do you abide by the size and bag regulations for kob?

□ yes □ no □ refused to answer

C1. The new regulations regarding size and bag limits are necessary.

□ agree □ disagree □ don't know

2. There is no need to worry about the kob stocks, there are lots left.

□ agree □ disagree □ don't know

3. Something needs to be done to facilitate the recovery of kob.

□ agree □ disagree □ don't know

D. Stock enhancement of kob

At the moment kob stocks are sitting at around 4% of what they used to be, and the current management is not facilitating their recovery.

Stock enhancement is a management method whereby kob will be grown in captivity in a hatchery until a certain size and then released into the wild to boost the stocks. This will result in an increased population and eventually the full recovery of the species so that it may be harvested sustainably.

The benefit to you as an angler would be that kob catches would become more frequent. Although the daily bag limit will not be increased, it may be attained more often.

But stock enhancement is expensive, so fishing licences would have to be increased.

Now I am going to suggest an amount by which licence fees would increase in order to pay for stock enhancement. The amount may sound much too low or much too high to you. It is just a starting point and you can tell me what the maximum amount you would be willing to pay would be.

Now, considering your annual income and expenses, would you be willing to pay and extra R 40 per year to support stock enhancement? This means that the yearly licence fee would increase to R 100.

□ yes □ no

1. What is the maximum additional amount you would be willing to pay in increased licence per year?

R_____________

2. How sure are you that you would really be willing to pay the amount that you mentioned? (read the options)

□ not at all sure □ fairly sure □ very sure □ don't know

E. (Only for those willing to pay for increased fishing licence)

1. Why are you willing to pay extra for a licence fee?

□ I may be catching more fish (personal gain)

□ Concerned about conservation

□ I'll pay for a fishing licence no matter how much it costs (within reason) because I want to fish.

□ I like to support a good cause

□ I like to know that future generations will be able to catch kob

□ Other______________________________ (specify)

(Only for those not willing to pay for increased fishing licence)

2. I am going to suggest some reasons why you might not be willing to pay the increased fee. Stop me when I say something that you agree with: (read the list).

□ Don't have enough money

□ Don't believe the government will use the money for stock enhancement

□ Don't believe I will catch more fish, or that stock enhancement will work

□ I don't fish very much anyway

□ I don't target kob

□ It doesn't matter if the kob stocks become extinct

□ Don't support the idea of stock enhancement (give reason):

____

____

____

F1. Please stop me when I mention your age group

□ 18-25 □ 26-35 □ 35-50 □ 51-65 □ >66

2. What is your race group? (only ask if not obvious)

□ Black □ coloured □ white □ Indian □ Other____________

3. Are you male or female? (only ask if not obvious)

□ Male □ Female

4. What is your highest level of education?

□ No schooling

□ Primary school

□ High School

□ Matric

□ Technikon

□ University

□ Other_________________________ (specify)

5. What is your job at the moment?

□ Management

□ Professional

□ Employee

□ Technical/Trade

□ Self-employed

□ Labour

□ Other________________________ (specify)

□ Unemployed

6. What is your monthly household income (after tax)? R____________

7. How many people in your household? ______________

Thanks so much for you time and help. Is there anything that you would like to know about this study?