PHYSIOLOGICAL RESPONSE OF SMALLMOUTH YELLOWFISH TO ANGLING: IMPACT OF ANGLING DURATION, FISH SIZE, FISH AGE, SEXUAL MATURITY, AND TEMPERATURE

Report to the
WATER RESEARCH COMMISSION

by

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WRC Report No. KV 285/11
ISBN 978-1-4312-0176-1

October 2011

The publication of this report emanates from a WRC project titled *Physiological response of smallmouth yellowfish to angling: impact of angling duration, fish size, fish age, sexual maturity, and temperature* (WRC Project No. K8/794).

EXECUTIVE SUMMARY

Freshwater angling activities have become an important recreational activity for people around the globe, bolstering both regional and national economies. A portion of the captured fish is sometimes kept by anglers, but many of them immediately release all of the fish that they catch back into their natural environment. This practice of “catch-and-release” (C&R) fishing is growing as a proportion of total fishing in southern Africa and is widely promoted by international and local angling fraternities, such as the Yellowfish Working Group. This practice is considered to be a very popular conservation strategy as well as a fisheries sustainable management tool, due to the assumption that no serious harm comes to pass on the fish that are being caught. There are only five species of fish for which there is an acceptable understanding of the effects that C&R angling poses to them. These species include largemouth bass *Micropterus salmoides*, walleye *Sander vitreus*, rainbow trout, *Onchorhynchus mykiss*, striped bass *Morone saxatilis* and Atlantic salmon *Salmo salar*. As such no information pertaining to the effects of C&R on local species exists. Two main factors that play a role in the effect of C&R on game fish include the age (size) of the fish angled as well as the reproductive state of the fish. These factors play an important role, since it has been shown that older (larger), sexually mature fish preparing for spawning are more severely affected by C&R sport angling than young, immature fishes of the same species. Therefore the study of the physiological affect of angling on a specific species will only have true application value if information pertaining to the variables of the age and reproductive state of that species are additionally considered.

The Orange-Vaal River system is the natural distribution range of *L. aeneus*. This species typically occupies slow to fast flowing habitats in predominantly the mainstream sections of rivers but also thrives in dams. *Labeobarbus aeneus* is not only renowned for its angling prowess and maintains the larger portion of a 133 million Rand industry in South Africa, but is also a highly sought after protein source by sustenance anglers in many rural communities. The practice of C&R of yellowfish in South Africa is currently considered to be an ecologically sustainable practice. Internationally however, this practice has received some criticism, and in many instances is considered to be detrimental to the long-term viability of specifically sensitive game fish populations. The ultimate success of C&R angling thus depends on ensuring high release survival rates by minimising handling, injury and mortality of caught individual fish.

In a recent review on C&R recreational angling, it is argued that a goal of conservation science and fisheries management should be the creation of species-specific guidelines for C&R practices. These guidelines must take into account the inter-species diversity of fishes and variation in angling techniques. As recreational angling continues to grow in popularity, expanding to many developing countries, it is important that data appropriate for specific fish and fisheries are available. The species specific data needed includes information on the:

- physiological effect of angling on the target species as well as all the different factors that influence the release mortality of that species,
- reproductive size (age) and reproductive period and
- population structure and dynamics of the targeted species.

In the light of the above information, angling stress has been shown to affect fish differently under various conditions, such as time of year (water temperature, spawning period) and fish size and/or age. It is thus of importance to have knowledge of the fish's biology to understand the threats that angling may pose to the individual and populations. To understand the biology of fishes, various parameters such as age, growth and sexual maturity (gonadal development) should be studied. In light that this information is extremely important for the development of proper C&R guidelines for freshwater sport fishing as well as the management of fish stocks, this project on a popular African freshwater angling species, *Labeobarbus aeneus* (smallmouth yellowfish) was undertaken.

To determine the physiological response of the smallmouth yellowfish (n=96), data was collected from June 2008 through to December 2008 (Vaal River). Fish were collected using standard angling and fly-fishing techniques, anaesthetised in clove oil and blood drawn from the caudal veins; thereafter fish were weighed and measured, revived and released. To serve as reference data, randomly selected fish were kept for 72 h in pools filled with river water. These fish were then anesthetised and blood was drawn from them again for a relative reference value.

Blood plasma was analysed for concentrations of glucose, cortisol and lactate to determine the effects of angling duration, fish size, and water temperature. Larger fish were shown to be angled for a longer duration compared to smaller fish. Levels of glucose, at times, were affected by water temperature (influenced by time of year). Plasma glucose concentrations in *L. aeneus* decreased with greater angling durations. Few individuals (n=12) showed significantly increased plasma cortisol concentrations. Lactate concentrations were found to

increase significantly above reference values in *L. aeneus* angled for > 1 min. Results from the reference fish indicated that baseline levels cortisol and lactate in *L. aeneus* were restored within 72 h of capture.

The relative ages of the *L. aeneus* were determined with the aid of scales and asteriscus otoliths. Males and females were found throughout the age classes. Male and female *L. aeneus* had longevities of 19 years and 15 years respectively. *Labeobarbus aeneus* males matured at a fork length (FL) of 289 mm and females matured at 367 mm FL, corresponding to relative ages of 4 and 6 years respectively.

This is the first study of its type reporting on the physiological response of the Vaal-Orange smallmouth yellowfish, while the age of various *L. aeneus* populations has been studied, this is the first time that otoliths were used for age determination in the Vaal River population.

The original aims proposed to test the hypotheses established for this study were to carry out assessments of the effects that selective angling activities (C&R) may pose to populations of *Labeobarbus aeneus* from the Vaal River, South Africa, to determine age, growth and size at maturity for this population. These aims were all achieved and this report presents the approach adopted outcomes, conclusions and recommendations made pertaining to the physiological response of smallmouth yellowfish to angling.

ACKNOWLEDGEMENTS

The authors would like to thank the following people and institutions for support during this project:

- Water Research Commission (WRC) for providing funding for this research on *L. aeneus* in the Vaal River (WRC PROJECT no. K8/794).
- Kobus Fourie and his staff at Elgro River Lodge for providing us with accommodation, as well as organising the fly-fisherman that enthusiastically took part in this study with much enthusiasm.
- Dr Olaf Wey (SAIAB) and Dr Deon Durholtz (MCM) for valuable assistance with specifically the aging section of this project.
- Liesel Whitlow for all your help in the lab with the blood plasma biomarker analysis.
- North-West DACERD (000113 NW -08) and Free State DETEA (HK/P1/10965/001) for the provision of the relevant permits.
- The University of Johannesburg's Centre for Aquatic Research for logistical support throughout this study.

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1 INTRODUCTION

Freshwater angling activities have become an important recreational activity for people around the globe, bolstering both regional and national economies. A portion of the captured fish is sometimes kept by anglers, but many of them immediately release all of the fish that they catch back into their natural environment (Cooke and Suski, 2005). This practice of "catch-and-release" (C&R) fishing is growing as a proportion of total fishing in southern Africa and is widely promoted by international and local angling fraternities, such as the Yellowfish Working Group. This practice is considered to be a very popular conservation strategy as well as a fisheries sustainable management tool, due to the assumption that no serious harm comes to pass on the fish that are being caught (Bartholomew and Bohnsack, 2005). According to Cooke and Suski (2005) there are only five species of fish for which there is an acceptable understanding of the effects that C&R angling poses to them. These species include largemouth bass *Micropterus salmoides* (Lacepède, 1802), walleye *Sander vitreus* (Mitchill, 1818), rainbow trout, *Onchorhynchus mykiss* (Walbaum, 1792), striped bass *Morone saxatilis* (Walbaum, 1792) and Atlantic salmon *Salmo salar*, Linnaeus, 1758. As such no information pertaining to the effects of C&R on local species exist. Two main factors that play a role in the effect of C&R on game fish include the age (size) of the fish angled as well as the reproductive state of the fish. These factors play an important role, since it has been shown that older (larger), sexually mature fish preparing for spawning are more severely affected by C&R sport angling than young, immature fishes of the same species (Cooke and Suski, 2005). Therefore the study of the physiological affect of angling on a specific species will only have true application value if information pertaining to the variables of the age and reproductive state of that species are additionally considered.

A fish's response to a stressor in the environment can occur at many levels of organisation from the cellular, to individual organisms, to population (Iwama et al., 2004) and if we can measure the response at a given level of organisation we can use it as an indication of the response of the individual to a stressor. A stress response of a living organism through the sympathetic division of the autonomic nervous system is an integral component of the physiology of the organism. Three categories can be distinguished within the general stress response of fish, firstly an initial neuroendocrine response (Gamperl et al., 1994) resulting in the release of stress hormones, such as catecholamines and cortisol into circulation (Iwama et al., 2004). Secondly, perturbations in the organism's biochemistry and physiology may occur, which are largely influenced by the aforementioned stress hormones. These result in blood chemistry and haematological changes (Barton et al., 2002, Iwama et al., 2004), such

as increased glucose and lactate levels, which result from adjustments to metabolism, respiration, acid-base balance and the immune function of the individual fish (Barton et al., 2002). Finally, the tertiary response, which represents changes to individuals and populations and diverts energy away from essential life processes such as growth and reproduction (Barton et al., 2002, Iwama et al., 2004). For game fish one such potential stressor is capture by sport anglers. The increased popularity for freshwater game-fish angling in southern Africa, and worldwide, highlights the importance in elucidating the response of these fishes to sport angling stress. One of these species, the Orange-Vaal smallmouth yellowfish (hereafter referred to as smallmouth yellowfish), *Labeobarbus aeneus* are recognised and widely promoted (Bloomer et al., 2007) as a popular sport angling fish (Groenewald, 1958; Jubb, 1961; 1962; 1973). They are considered the most important angling fish species in the Orange-Vaal River system (Gaigher, 1976).

1.1 Orange-Vaal smallmouth yellowfish

The Orange-Vaal River system is the natural distribution range of *L. aeneus* (Skelton 2003). This species has however been translocated or introduced to many other river systems in South Africa, such as the Great Fish River (Weyl et al., 2009), the Gouritz River, the Olifants River, the Limpopo System, the Great Kei System and the Swart Kei System where it is classified as an invasive species (Jubb, 1961/62; Weyl et al., 2009). This species typically occupies slow to fast flowing habitats in predominantly the mainstream sections of rivers but also thrives in dams (Jubb, 1961/62; Jubb and Farquharson 1965; Jubb 1972a; Mulder 1973; Cambray and Jubb 1977; Skelton and Cambray 1981; Hocutt and Skelton 1983; Gaigher and Fourie 1984; Tómasson et al. 1984; Dörgeloh 1994, 1996). Although this species adapts readily to still water currents, selected habitats are generally considered to be required for them to breed successfully (Mulder 1973; Tómasson et al., 1984). *Labeobarbus aeneus* is not only renowned for their angling prowess (Groenewald, 1958) and maintains the larger portion of a 133 million Rand industry in South Africa, but they are also a highly sought after protein source by sustenance anglers in many rural communities (Bloomer et al., 2007, Brand et al., 2009).

1.2 Catch and release angling

Importance of effective C&R practices

The practice of C&R of yellowfish in South Africa is currently considered to be an ecologically sustainable practice (pers. comm.: De Villiers[1]). This has resulted in ecosystem managers allowing yellowfish, regardless of their endangered status, to be angled (e.g. Bells Smallmouth and Largemouth yellowfish fly fishing festivals). Internationally however, this practice has received some criticism, and in many instances is considered to be detrimental to the long-term viability of specifically sensitive game fish populations (Brobbel et al*.,* 1996, DuBois and Dubielzig, 2004, Bartholomew and Bohnsack, 2005, Meka and McCormick, 2005). Some of these publications further indicate that the practice of C&R methodologies, if not carried out correctly, may lead to a high percentage of fish mortalities primarily due to the induction of excessive stress during the catching procedure (Meka and McCormick, 2005). The ultimate success of C&R angling thus depends on ensuring high release survival rates by minimising handling, injury and mortality of caught individual fish (Bartholomew and Bohnsack, 2005). This should result in acceptable survival rates being maintained, as well as the released individuals taking part in successful reproduction (Bartholomew and Bohnsack, 2005).

Sub-lethal physiological effects

Effects associated with C&R include a suite of sub-lethal physiological, behavioural and fitness impairments that can arise from C&R angling. Cooke & Suski, (2005) noted that these sub-lethal effects may range from osmoregulatory imbalances, depletion of energy stores, cardiovascular disturbances, damage of the tissues, hormonal changes as well as a build up of metabolic wastes. The reproductive success of a fish is often altered after it has been caught or if it has been exposed to exhaustive exercise (Pankhurst and Dedual, 1994; Tomasso et al., 1996; Meka and McCormick, 2005). Such alterations have been observed in nesting male largemouth bass when angled (**ref**). These males, when returned to the water, may not return to their nests containing their eggs, they may even exhibit movement impairments or decreased fitness as a result of endocrine and hormonal alterations, or the eggs that the male was guarding may be eaten by predators (Suski et al., 2003).

[1] Pierre De Villiers: Chairman of the Orange Vaal Yellowfish Conservation and Management Association.

Currently, three informal approaches have been established in South Africa to minimise the impact of angling activities on freshwater fish populations namely: (1) minimise air exposure of the captured fish, (2) avoid angling during extreme environmental conditions such as extreme water temperatures and (3) refrain from angling fish during spawning periods. These approaches provide some level of protection to all species, but have their limitations.

Post-release mortality

C&R angling that result in the mortality of the individual can be considered to be the most extreme impact of angling to a game fish population. Most fish which die from angling stress tend to do so some time after being released and as such most anglers and fisheries managers assume that mortality arising from C&R angling is negligible (Cooke and Suski, 2005). Although mortality rates may be low in some species, others may indeed experience high, often unnoticed mortality levels. Muoneke and Childress (1994) reported that mortality rates, due to excessive stress for released fish ranged from 0 to 89% across many marine and freshwater species. Mortalities have been observed to occur between hours to days after capture (Meka and McCormick, 2005). In their review of C&R angling mortalities, Bartholomew and Bohnsack (2005) found that mortality was greater for larger individuals of non-anadromous trout and striped bass than smaller individuals, however, smaller individuals of lake trout and Chinook salmon were found to have higher mortality than the larger individuals. Similarly, Thorstad et al. (2003) found that Atlantic salmon caught in Norway also showed increased lactate and calcium levels with increasing body size. Other factors affecting the survival of fish have been assumed to be the so called “playing time” of the fish (Cooke and Suski, 2005), handling time and the angling experience of the angler. These factors have also been found to affect recovery time of the fish (Meka and McCormick, 2005). For striped bass, neither playing time nor handling time affected mortality of individuals (Tomasso et al., 1996), these factors however caused significant influences in plasma lactate and cortisol of wild rainbow trout (Meka and McCormick, 2005).

Factors affecting post-release mortality

Many factors have an effect on the mortality of angled fish, one such factor, as mentioned above, is angling duration. It is widely accepted that the angling duration is directly correlated to the magnitude of the physiological disturbance (Cooke and Suski, 2005; Meka and McCormick, 2005). Thorstad et al. (2003) found that with increased angling duration, Atlantic salmon had increased plasma lactate, and Pankhurst and Dedual (1994) also found that longer angling times resulted in increased levels of plasma lactate and gonadal steroids

in the blood. Similarly Meka and McCormick (2005) found that with increased angling duration wild rainbow trout had increased plasma lactate and cortisol levels; in addition, increased plasma glucose and lactate levels were found in striped bass that were angled for longer durations (Tomasso et al., 1996). Muscle lactate concentrations were found to increase 10 fold in wild Atlantic salmon that were angled to exhaustion (Wilkie et al., 1996). Cooke and Suski (2005) noted that angling events should be shortened, and agree that this can be done by using more appropriate gear and equipment.

Water temperature has also been found to have a significant effect on mortality as well as a significant physiological impact on the fish (Brobbel et al., 1996; Thorstad et al., 2003; Cooke and Suski, 2005; Meka and McCormick 2005). Greater mortality in higher water temperatures have been found for striped bass (Tomasso et al., 1996), rainbow trout, cutthroat trout *Oncorhyncus clarkii clarkii* (Richardson, 1836), and Atlantic salmon (Brobbel et al., 1996). Levels of plasma cortisol, lactate and glucose in wild rainbow trout were shown to be significantly influenced by warmer water temperatures (Meka and McCormick, 2005). This effect of higher temperatures results in less oxygen availability in the water, and thus causing a greater oxygen debt in the angled individual. Higher water temperatures result in increased levels of plasma glucose, lactate and cortisol (Tomasso et al., 1996) in some fish species. Cooke and Suski (2005) showed that angling duration and handling time should be minimized, and ideally fishing should be prohibited during the warmest water temperatures during the year. High water temperatures, as high as 30ºC, were however found not to influence post release mortality of the cichlids species nembwe *Serranochromis robustus* (Boulenger, 1896), and threespot tilapia *Oreochromis andersonii* (Castelnau, 1861) in the Upper-Zambezi River (Thorstad et al., 2004).

Air exposure time is another factor that is harmful towards fish (Thorstad et al., 2003), and it has been found that the acid/base balance of fish is severely affected by air exposure time, and this may even result in permanent tissue damage in some fish species (Cooke and Suski, 2005). Mortality rates are often underestimations of the actual mortality rates (Bartholomew and Bohnsack, 2005). Recovery periods of fish are extended (Brobbel et al., 1996; Bartholomew and Bohnsack, 2005) following heavy exercise and during this period the fish may show different behavioural patterns and become more susceptible to predation (Bartholomew and Bohnsack, 2005). Many studies have noted that physiological disturbances due to exercise may lead to the delayed mortality of angled fish some time after release. It has also been speculated that this delayed mortality is due to the physiological disturbance occurring in the white muscle of the fish (Brobbel et al., 1996).

These physiological disturbances are attributed to high plasma levels of glucose, lactate and cortisol (Pankhurst and Dedual, 1994; Meka and McCormick, 2005). It was found that plasma lactate and glycogen levels in Atlantic salmon angled from Miramichi river, rose significantly after being angled, and lactate continued to rise for a further 2 hours after angling, and took 12 hours for resting levels to be attained (Brobbel et al., 1996). Similarly levels of plasma lactate and gonadal steroids in rainbow trout angled in New Zealand were found to increase over the next hour after capture. A further 24 hours were necessary for recovery to take place and plasma lactate and gonadal steroid levels to stabilise again (Pankhurst and Dedual, 1994). Meka and McCormick (2005) found that plasma lactate and cortisol levels in wild rainbow trout started rising within 2-3 minutes of the onset of capture and that maximum levels were found to occur within 5-6 minutes.

In a recent review on C&R recreational angling, Cooke & Suski (2005) argue that a goal of conservation science and fisheries management should be the creation of species-specific guidelines for C&R practices. These guidelines must take into account the inter-species diversity of fishes and variation in angling techniques. As recreational angling continues to grow in popularity, expanding to many developing countries, it is important that data appropriate for specific fish and fisheries are available. The species specific data needed includes information on the:

- physiological effect of angling on the target species as well as all the different factors that influence the release mortality of that species,
- reproductive size (age) and reproductive period and
- population structure and dynamics of the targeted species.

In the light of the above information, angling stress has been shown to affect fish differently under various conditions, such as time of year (water temperature, spawning period) and fish size and/or age. It is thus of importance to have knowledge of the fish's biology to understand the threats that angling may pose to the individual and populations. To understand the biology of fishes, various parameters such as age, growth and sexual maturity (gonadal development) should be studied.

1.3 Fish age and growth

Ageing and population growth dynamics are essential parameters in fisheries management (Booth et al., 1995a; Booth and Merron, 1996; Campana, 2005; Potts and Cowley, 2005)

and are used to evaluate the state of exploited species (Allain and Lorance, 2000). Annual age and growth studies provide knowledge and form the basis for comparative growth rates and life history studies (Campana, 2005).

Ageing structures

Of the many bony structures currently used to age fish, otoliths and scales are the most commonly used (Booth et al., 1995a; Wischniowski and Bobko, 1998; Campana, 2001). Due to their easy collection and non-lethality in sampling, scales have traditionally been the ageing structure of choice (Wischniowski and Bobko, 1998). Scale estimates should however be viewed with caution, particularly if these estimates are to be used in fishery management (Booth et al., 1995a). In temperate water regions deposition patterns found on these hard structures are the most visible, and thus directly correlated to seasonal changes in environmental conditions (Wischniowski and Bobko, 1998). This is most likely due to changes in temperature (Beamish et al., 2005). Otoliths were found to be used over the broadest age range in many fish species (Campana, 2001). In fish, the otoliths are usually found in the auditory organs, and typically three otoliths are found per auditory organ (Jones and Hynes, 1950; Wischniowski and Bobko, 1998). These three otoliths are known as the sagitta, lapillus and the asteriscus, and are respectively largest to smallest in non-ostariophysean fish; the largest otolith, the sagitta is most commonly used when ageing fish (Jones and Hynes, 1950; Booth and Merron, 1996; Wischniowski and Bobko, 1998; Beamish et al., 2005). In ostariophysean fish such as *H. vittatus* and *L. aeneus* the asteriscus is the largest and the sagitta the smallest. The absolute otoliths size tends to increase with increasing fish size; however in relative terms larger fish tend to have “relatively smaller” otoliths compared to smaller fish (Jones and Hynes, 1950).

When viewed under a microscope with transmitted light, otoliths normally show alternating bands commonly named opaque and transparent bands/sections (Jones and Hynes, 1950; Wischniowski and Bobko, 1998; Potts and Cowley, 2005), or opaque and hyaline zones (Booth et al., 1995a; Booth and Merron, 1996). However, when viewed under reflective light against a dark background the opaque zones appear white and the hyaline zones appear black (Jones and Hynes, 1950; Wischniowski and Bobko, 1998). Opaque bands are generally deposited during winter months due to reduced metabolic activity associated with lowered water temperatures, whilst the wider transparent bands are deposited during the summer months (Wischniowski and Bobko, 1998). Other factors such as a shift in energy allocation during spawning can also result in the deposition of additional bands (Kanyerere et al., 2005; Weyl and Booth, 1999).

In otoliths the combination of the two bands, namely the opaque and transparent bands, are referred to as an annulus (Wischniowski and Bobko, 1998). The counting of annuli on otoliths and circuli on scales are the most common ways of determining fish age (Wischniowski and Bobko, 1998). In many studies otoliths have been found to be the only acceptable method for the ageing of various fishes as this is considered to be a more reliable method (Jones and Hynes, 1950; Allain and Lorance, 2000; Campana, 2001). For the interpretation of annuli certain areas on the reading surface are favoured in view of the fact that some of the annuli are more distinct in these areas. However, reading planes are both species and age dependant (Wischniowski and Bobko, 1998).

Ageing techniques

Due to the otoliths being internally situated they are not exposed to external elements and as a result they present a good permanent pattern of fish growth (Wischniowski and Bobko, 1998). Otoliths show continuous growth unlike scales, spines and vertebrae and are metabolically inert (Campana, 1999; Campana and Thorold, 2001). This contributes to the reliability of the used of this hard structure to age fishes (Campana, 2001). Otoliths can be read using several different techniques. These methods include reading them whole (Jones and Hynes, 1950; Allain and Lorance, 2000), the break and burn technique (Beamish et al., 2005), similarly the break and baking technique, thin sectioning of the otolith through the nucleus (Booth et al., 1995a; Wischniowski and Bobko, 1998; Allain and Lorance, 2000; Beamish et al., 2005) and lastly burning the otolith and sectioning thinly (Booth and Merron, 1996; Weyl and Hecht, 1998). Each of these techniques have their advantages (Wischniowski and Bobko, 1998).

Research has shown that more annuli are visible in otoliths when using sections than using whole otoliths, and sections are generally more accurate when estimating fish age (Booth and Merron, 1996; Dwyer et al., 2003; Brouwer and Griffiths, 2004). The use of whole otoliths therefore often results in an under ageing of the fish in question (Dwyer et al., 2003; Brouwer and Griffiths, 2004). It has been found that with practice most otoliths could be interpreted (Kimura et al., 2006), and that auxiliary rings more commonly known as “false” rings could be distinguished from the major rings or “true” rings (Jones and Hynes, 1950; Brouwer and Griffiths, 2004).

Fish growth patterns

Individual fish have been found to have different growth rates during spawning periods, whereas at other times of the year fishes tend to follow a trend in their growth (Jones and Hynes, 1950). During spawning periods fish growth tends to slow and results in the deposition of growth checks and increments (Booth et al., 1995a; Booth and Merron, 1996; Potts and Cowley, 2005). Other factors which could cause slow growth and result in deposition of opaque zones in fish otoliths include, maturation (Dwyer et al., 2003), sex reversal, and various changes in environmental conditions (Booth et al., 1995a; Booth and Merron, 1996; Potts and Cowley, 2005). It has been shown that most fishes have a high growth rate in their first year (Jones and Hynes, 1950; Booth et al., 1995a) and even up until sexual maturation has been reached (Booth et al., 1995a; Booth and Merron, 1996; Dwyer et al., 2003). According to Wischniowski and Bobko (1998) growth in both fish and otoliths is faster in summer periods compared to winter periods. Male and female fish do not necessarily have corresponding growth rates and for most species females generally tend to grow faster than males (Beamish et al., 2005; Yamaguchi et al., 2006). Fish growth is most commonly plotted using the 3 parameter von Bertalanffy growth curve (Beamish et al., 2005). These growth curves are based on length at age data from all age readings (Dwyer et al., 2003).

Otolith research

Campana (2005) found that otolith orientated research papers are generally dominated by annual age and growth studies. However otoliths can be used for more than just an indicator of age, it can be used to determine larval fish ecology, identification of species, population dynamics, tracer applications and environmental reconstructions (Campana, 2005). After reviewing 862 papers, Campana (2005) found that 24% of these papers involved otolith microstructure – to identify daily growth increments, for the validation of the first formed annulus. A further 23% of these papers were concerned with annual age and growth – annual growth patterns are used to estimate the population growth rate and longevity of different fish species. Age validation and ageing method comparisons were found in 10% of the papers – such as comparing whole otoliths with sectioned otoliths and otoliths with scales, population dynamics (Campana, 2001; Campana, 2005); as well as species identification (use of otoliths from stomachs or droppings to identify predated species) each formed another 6% of the papers, respectively. The remaining papers consisted of otolith allometry (4%), tracer applications (12%), mass marking (4%), trace elements (4%),

methods (3%), physiology (2%), hearing and balance (1%), isotopes (1%), environmental reconstruction (<1%) and fossils (<1%).

Accuracy and precision of age estimates

Validation of the method used to determine fish age, be it counting annuli on otoliths or circuli on scales, is a necessary element in fisheries management (Campana, 2001). Validation of an ageing method can be done in various ways, such as determining the age at first increment (Campana, 2001; Dwyer et al., 2003; Brouwer and Griffiths, 2004), using flourochrome marking to verify the periodicity of zone formation (Brouwer and Griffiths, 2004; Potts and Cowley, 2005), marginal increment analysis (Booth et al., 1995a; Campana, 2001; Dwyer et al., 2003; Brouwer and Griffiths, 2004; Beamish et al., 2005; Kimura et al., 2006) and bomb radiocarbon assays (Campana, 2001; Dwyer et al., 2003). Oxytetracycline (OTC) is often used to validate zone formation in otoliths (Campana, 2001; Brouwer and Griffiths, 2004; Potts and Cowley, 2005). Validation of ageing increments has been done on many fish species namely: roman seabream *Chrysoblephus laticeps* (Valenciennnes, 1830), santer seabream *Cheimerus nufar* (Valenciennnes, 1830), poenskop seabream *Cymatoceps nasutus* (Castenau, 1861) and galjoen *Dichistius capensis* (Cuvier, 1831) although all these species are from southern Africa they are marine species. Examples of freshwater populations for which age validations have been done include sharptooth catfish *Clarias gariepinus* (Burchell, 1822) (Weyl and Booth, 2008), Vaal-Orange smallmouth yellowfish *L. aeneus* (Ellender, 2008; Weyl et al., 2009) and silver catfish *Schilbe intermedius*, Rüppell, 1832 (Khumalo 2006). Many freshwater species and their subsequent populations are still lacking age validation. Two sources of error have been found to accompany age determination and can have serious influences on age-structured calculations (Campana, 2001). These errors are firstly a process error, in most cases this type of error is biased towards under or over-ageing, and secondly an error as a result of an element of subjectivity that forms an essential component of all age estimations, this type of error originates from the preparation and interpretation of the features of calcified structures such as otoliths (Campana, 2001). Precision provides a means of determining the ease with which age determinations can be inferred from hard ageing structures and a means of assessing the inter- and intra-individual reproducibility of age determinats (Campana, 2001). Precision is defined by Campana (2001) as “the reproducibility of repeated measurements on a given structure, whether or not those measurements (age readings) are accurate”. Average percent error (APE) and coefficient of variance (CV) are two statistically sound and widely used measures of ageing precision (Campana, 2001).

Despite the known importance of *L. aeneus* little has been published on many aspects of the ecology and biology of this species, specifically on its age structure in the Vaal River system, where the only published information dealing with smallmouth yellowfish from this locality is an age and growth study based on lateral line scales (Mulder, 1973). Ageing is an essential parameter used in fisheries management (Booth et al., 1995a; Booth and Merron, 1996; Campana, 2005; Potts and Cowley, 2005) and in population dynamic studies to evaluate the state of exploited species (Allain and Lorance, 2000). According to Griffith (1975), the management of many tropical and sub-tropical freshwater fish, has been hindered by a lack of knowledge on their age structure. This study focused on precision of accurate annuli counts rather than the accuracy of counted annuli to validated age studies that are not available. The periodicity of ring formation in smallmouth yellowfish otoliths from Lake Gariep, Orange River (Ellender, 2008) as well as the Great Fish River and Glen Melville Reservoir (Weyl et al., 2009) have however been validated. These authors used chemical marking of the otoliths by oxytetracycline (OTC) and edge analysis, respectively to validate age. The periodicity of ring formation in these populations was found to be a single growth ring per year, i.e. an annulus. Since environmental factors such as seasonal water temperature fluctuations and variation in flow rates are similar for the Orange, Great Fish and Vaal Rivers the growth zones counted in this study (Vaal River) will thus be considered annuli. Since the only study on Vaal River smallmouth yellowfish age used lateral line scales for age determination an additional aim of this section of the MSc project is to determine the most suitable method for estimating the age of Vaal River *L. aeneus* and then use the most accurate of the methods to provide information on growth rates as well as size and age at sexual maturity of both sexes of this species.

1.4 Hypotheses, aims and objectives

It is thus clear from the available literature that there is a paucity of knowledge on the physiological response of southern African freshwater game fishes to angling as well as the age and growth of these predatory fishes. In light that this information is extremely important for the development of proper C&R guidelines for freshwater sport fishing as well as the management of fish stocks, this project on a popular African freshwater angling species, *Labeobarbus aeneus* (smallmouth yellowfish) was proposed. The following hypotheses, aims and objectives were established for this project.

Hypotheses

The hypotheses to be tested in this study include:

- Blood plasma lactate, glucose and cortisol levels can be used as bioindicators of physiological angling stress in *L. aeneus*.
- Larger (older) fish experience longer angling durations and experience greater angling stress in comparison to smaller individuals and thus standing less chance of survival.
- Increased angling duration results in a greater stress response, i.e. the longer the landing time and handling time the greater the stress response in *L. aeneus* that may be further exacerbated during spawning periods.
- During extreme high water temperatures the negative effects of angling become greater, i.e. increased water temperature results in a greater angling stress response.
- *L. aeneus* are fast growing, fast maturing and able to reproduce from early age.
- Age data obtained from scales do not reflect the true age of fish

Aims and Objectives

To test the various hypotheses aforementioned, the aim of this study is to carry out assessments of the effects that selective angling activities (C&R) may pose to a population of *L. aeneus* from the Vaal River, South Africa, as well as determining age, growth and size at maturity for this population. In order to meet these aims the following objectives were established:

- Assess the occurrence, locations, methods used, and specimens targeted in the catch-and-release angling activities of smallmouth yellowfish in their respective system.
- Assess the stress and related physiological effects, of C&R practices, smallmouth yellowfish during surveys carried out in 2008.
- During these surveys, carry out additional fish sampling techniques (gill nets, and angling) to capture individuals to undertake age determination assessments and gonadal development assessments.
- In the laboratories of the University of Johannesburg, assess the gonadal development of the fish sampled in this study by assessing the reproductive size (age) of the fishes in this system. Assess the age of captured specimens using otoliths and scales, and construct accurate weight, length and age growth curves. This is important in order to determine whether sex, sexual development and age have an influence on the physiological response of a fish to angling.
- Develop initial, sustainable, non-destructive, conservation friendly catch-and-release practice methodology for indigenous game fish in South Africa.

This report presents the approach adopted outcomes, conclusions and recommendations made pertaining to the physiological response of smallmouth yellowfish to angling.

2 MATERIALS AND METHODS

2.1 Study area

The catchment area of the Vaal River is roughly 192 000 km^2 (Braune and Rogers, 1987) and is the main water source for South Africa's central regions. The Vaal River originates in the lake Chrissie area of the Drakensberg escarpment, from here the river flows in a west-south-westerly direction until it meets and merges with the Orange River close to Douglas (Bertasso, 2004). The Vaal River is approximately 900 km in length (Braune and Rogers, 1987) and its major tributaries drain the Eastern Drakensberg, the Witwatersrand and the Maluti mountains. Along its length, the Vaal River contains several of South Africa's major dams, namely, Grootdraai, Vaal Dam, Vaal Barrage, Bloemhof, Vaalharts and Douglas Weir (Bertasso, 2004).

Within the Vaal River, typical *L. aeneus* habitat consists of pools, rapids and riffles (Mulder, 1973; Eccles, 1986; Skelton, 1993, De Villiers and Ellander, 2008). This species prefers areas where clear fast-flowing water with sand or gravel substrates (Mulder, 1973; Skelton, 1993; Dorgeloh, 1994; 1995). Adult and sub-adult *L. aeneus* utilise most of the available habitats in both running water and still water area of the Vaal River. Adult fish prefer riffles during the summer months where spawning and feeding takes place and then frequent deeper pools and backwater areas in the cooler months (De Villiers and Ellender, 2008).

A portion of the Vaal River that is known to have a large population of smallmouth yellowfish that are actively targeted by yellowfish dependant anglers (fly-fishermen) was selected as the study area for this study (Brand et al., 2009). The area considered is located approximately 30 km south of Potchefstroom on the boundary between the North West and Free State provinces of South Africa (Figure 1).

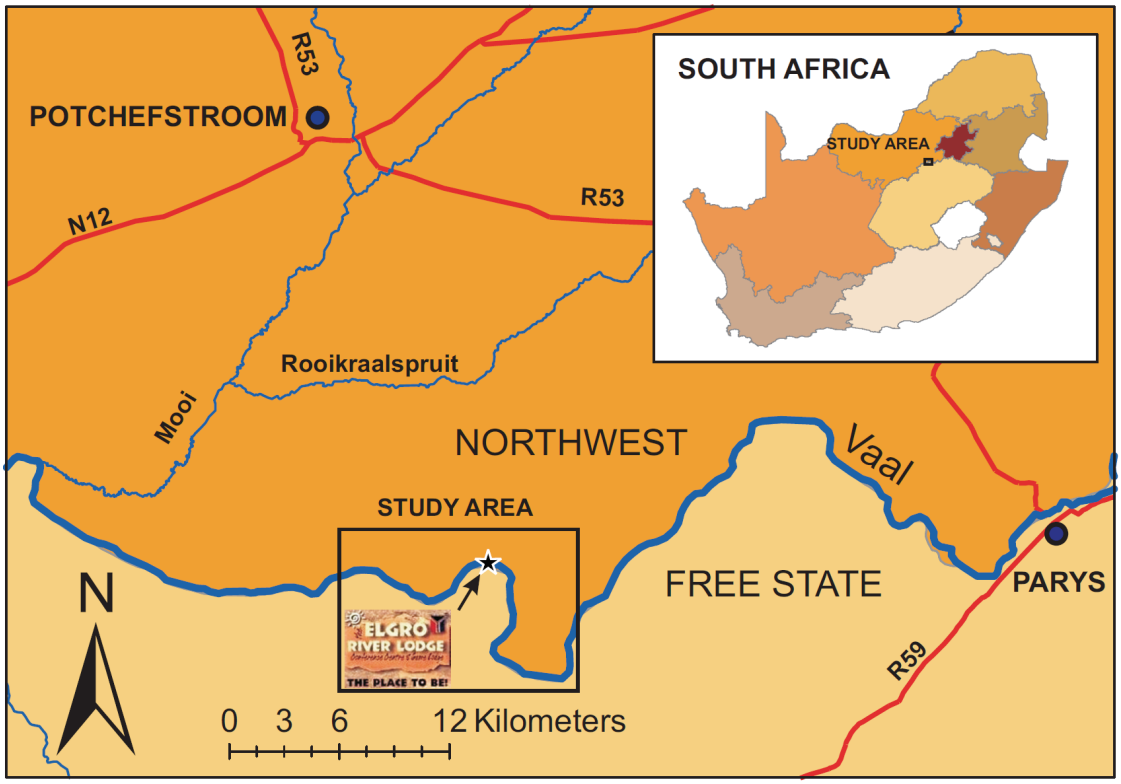

Figure 1: Map of the study area and surrounds in South Africa, the marker indicates the sampled area in the Vaal River.

The study area includes the Elgro River lodge that were used as a base of operations throughout the surveys (www:elgroriverlodge.co.za). The approximately 12 km of the Vaal River that was considered to contain many diverse habitat types shown to be utilised by *L. aeneus* (Figure 2). Furthermore established fly-fishing activities that target *L. aeneus* occur within this study area, including organised Windknot fly-fishing and conservation club activities and the annual Orange-Vaal River smallmouth yellowfish Bells Fraternity Fly-fishing Festival (www:elgroriverlodge.co.za and www.windknot.co.za).

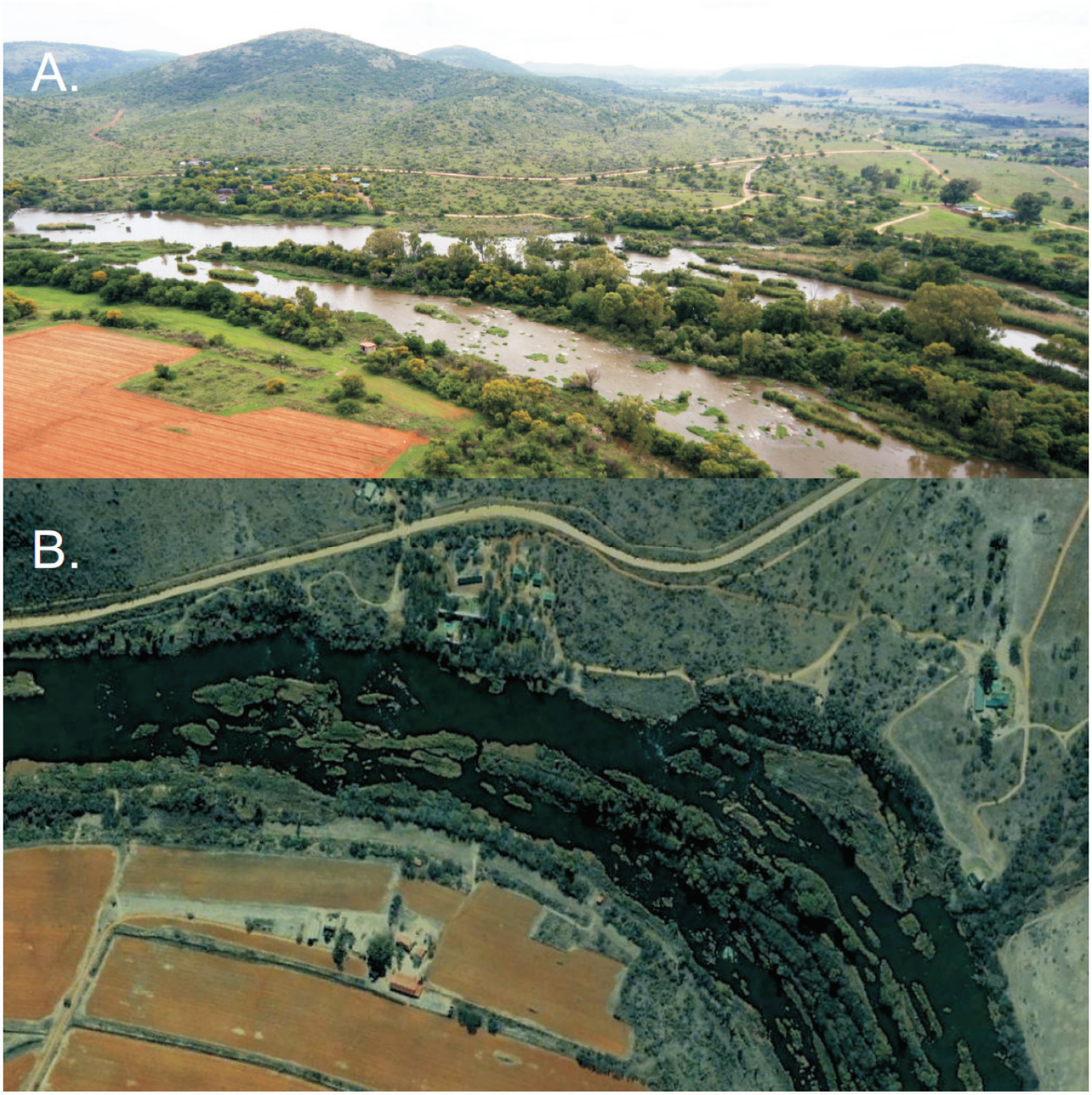

Figure 2: Photographs depicting sampling area in the Vaal River, a, b, c) satellite images (Google maps) showing the many islands and smaller channels in the area, d, e) photos showing the river as well as some of the surrounding vegetation.

2.2 Sampling

In this study a total of 96 *L. aeneus* were captured in 2008 from June (winter) to December (summer) by means of fly-fishing techniques in the middle reaches of the Vaal River (Figure 2) (North West Department of Agriculture, Conservation and Environment permit no. 000113 NW -08, and Free State Department of Tourism, Environmental and Economic affairs permit

no. HK/P1/10965/001). Fly-fishing techniques were selected for this study as is has been established to be the only approach that specifically targets yellowfish in the Vaal River (Brand et al., 2009). Fly fisherman were encouraged to fish as normal and the primary gear setup used during this study was a 5/6 weight rod fitted with floating line and a 2X monofilament tippet. Fishermen used a variety of flies that were either recommended by professional guides or tried and tested patterns that had been developed independently. The fishing areas were dependant on the time of year (and thus changes in water temperature). During the colder winter months (June to mid September) smallmouth yellowfish were targeted in deeper habitats such as pools. In the warmer months (September to December) shallower fast flowing habitats of the river, mainly riffles and rapids, were targeted (Figure 3a-g). When fishing the pools, anglers used inflatable boats (Figure 3a&b), and in shallower habitats anglers waded into habitats, no deeper than the mid-thoracic region. Anglers participating in this study consisted of a group of fly-fisherman with varying experience. The more experienced group of fisherman had been fishing for *L. aeneus* in this river system for more than 3 years (range 3 to 25 years), whereas the inexperienced anglers had < 2 years fishing experience. For each fish caught (Figure 3e-g)) the time taken to land the individual and the duration of the handling procedure (hook removal) were recorded. Landing time refers to the time from when the fish was hooked until it was landed (caught in the net); the handling time refers to the time from when the fish is netted by the angler, the hook removed (handling times were prolonged when anglers took photos with the fish they caught) and the fish was then placed into the anesthetising solution of water and clove oil. Landing and handling times for each fish were combined to calculate total angling time.

Anaesthesia and sampling procedure

Following capture, all fish were anaesthetised for 2 minutes in a 96 L container containing 50 L of fresh river water with a 32 mg/L concentration of clove oil solution [1:9 ratio of clove oil mixed with ethanol (Anderson et al., 1997; Meka and McCormick, 2005)]. Anaesthetised fish were removed from the container when signs of anaesthesia were observed and 2 mL of blood was drawn from the caudal vein using sterile 1 mL syringes and 1½ inch 21 gauge needles. Blood was immediately transferred to 4 mL heparinised vacutainers and kept cool until centrifugation to separate the plasma supernatant. Plasma was drawn and placed into 1½ mL eppendorf tubes, stored in liquid nitrogen in the field and then stored at -80°C on return to the laboratory, until analysis took place. In addition to the timings (landing and handling times), general fish characteristics (mass and various length measurements: standard length (SL), fork length (FL) and total length (TL)) were recorded after blood had

been drawn (Figure 3h-j). On completion of data collection, each fish was revived in flowing river water and released (Figure 3k).

Figure 3: Photographs depicting data collection procedure including; inflatable canoes fitted with containers (anaesthetisation) and used to carry sampling equipment (a&b). Wading fly-fisherman targeting *Labeobarbus aeneus* in shallow waters (c), fly-fisherman fishing from boat (d), angler with

his catch (e) and two more specimens landed by fisherman (f&g), collection of data (h, i, j) and revival and release of test organism (k).

2.3 Sub-lethal physiological effects

Plasma glucose and lactate were determined using Roche/Hitachi kit (model no. 11448668 216 [CV = 10.65%] and model no. 11822837 190 [CV = 9.12%] respectively, Mannheim, Germany). Plasma cortisol was determined through ELISA, using a research cortisol test kit (model no. 402710 [CV = 8.74%], Neogen Corporation, Lexington, Kentucky USA). The volumes required for the glucose and lactate analysis were adapted so that the reactions could take place in 300 µl microplate wells. Plasma cortisol was determined through ELISA, using a research cortisol test kit (Model no. 402710 [CV=9.09%], Neogen Corporation, Lexington, Kentucky USA). Plasma glucose, cortisol and lactate assays were run or analysed on a Biotek microplate reader at wavelengths of 540 nm (glucose) and 630 nm (cortisol and lactate). Concentrations were calculated from the absorbances obtained, by means of equations provided, for cortisol a standard curve (0-10 $ng.mL^{-1}$) was constructed. Physiological changes caused by both landing and handling times (form a part of exhaustive exercise and handling stress, respectively) have previously been shown in fish species (Meka and McCormick, 2005), these times were analysed separately as well as in combination. The time that fish were anaesthetised was not included during blood parameter analysis, as this time was kept constant (2 min) and clove oil is known to impose a negligible physiological effect with short exposure times (Wagner et al., 2002). The most influential factors contributing to changes in the physiological response was considered to be both landing as well as handling time, because of the exhaustive nature of hooking and landing processes (Booth et al., 1995b; Meka and McCormick, 2005).

Reference group

The reference group consisted of sixteen randomly chosen fish collected during the study by anglers. Following the initial blood drawing, fish were revived in fresh river water and kept in an aerated 96 L insulated container filled with fresh river water. These individuals were then transported to the field laboratory within 60 min and released into a 15 000 L aquarium (pool) containing fresh river water. A 10 000 $L.h^{-1}$ water pump was used to aerate the water and simulate current. Twenty percent of the water in the reference pool was replaced daily to maintain suitable water quality (Smit et al., 2009). The reference fish were allowed to recover for a 72 hour period (Gustaveson et al., 1991; Smit et al., 2009), to allow the physiological stress response from capture to reduce and emulate the condition of free

swimming unstressed fish. After 72 hours clove oil was added to the water to anaesthetise the fish and a further blood sample was taken and analysed using the approach previously described. Samples obtained were used as reference values to examine the differences with values attained from captured fish.

Data analysis

All data were statistically analysed using SPSS for windows v. 15 similar to the approach used by Smit et al. (2009). All descriptive data are reported as means ± Standard Deviation (SD). Pearson's Correlation Coefficient was used to examine the influence of fish mass on landing time. Fish were grouped by landing time which was divided into minute intervals (< 1 min, 1-2 min, 2-3 min, etc.) and also grouped according to the water temperature they were caught at (11°C, 19°C, 22°C and 27°C). A one-way ANOVA was used to examine the plasma cortisol, plasma glucose, and plasma lactate responses resulting from the different landing times and water temperatures. A one-way ANOVA was also used to determine whether the water temperature groups differed significantly with regard to the landing times of the captured fish in each group (Smit et al., 2009). Differences between groups were determined with the aid of an LSD *post-hoc* test.

2.4 Age, growth and sexual maturity

Sampling

A total of 193 *L. aeneus* were captured using standard gill nets (mesh size: 38 mm, 45 mm, 70 mm and 93 mm (stretched)), from August 2008 through to June 2009. Captured fish were transported to a field laboratory, weighed to the nearest 10 g using a digital lip-grip scale, measured to the nearest mm (TL, FL and SL) killed with an overdose of 2-phenoxy-ethanol anesthetic and dissected for the removal of gonads (Figure 4).

Figure 4: Photographs depicting collection of data for the age, growth and sexual maturity portion of the study including; a captured *Labeobarbus aeneus* (a), fish being weighed (b), general measurements of specimen being taken (c) and gonads in body cavity prior to removal (d) and removed gonads (e).

Gonad development

Ovaries and testes were removed from 96 smallmouth yellowfish (Figure 4d&e), stored in 10% neutral buffered formalin and transported to the University of Johannesburg's histopathology laboratory for analysis. All samples were prepared for histological analysis following standard techniques (Humason, 1962), sectioned (5 µm) and stained with Hematoxylin and Eosin. Stained slides were interpreted for sexual maturity using the approach presents in Table 1 under a Zeiss transmission microscope, using the gonadal reproductive stage determination index proposed by Schmitt and Dethloff (2000). The gonads were considered to be sexually mature when testes and ovaries had ratings of stages 1 and above or 2 and above respectively (Table 1). Immature ovaries contain only pre-vitellogenic follicles (Figure 5a&b). Immature testes contain no spermatozoa (Figure 5c&d). Mature ovaries have mid-vitellogenic, to late-vitellogenic follicles as well as spent follicles (Figure 5e&f). Mature testes contain spermatozoa in varying quantities (Figure 5g&h).

Table 1: Histological criteria (adapted from Schmitt and Dethloff, 2000) used in gonad staging of male and female *Labeobarbus aeneus* to determine when sexual maturity is reached.

Stage	Testis characteristics	Ovary characteristics
0	Undeveloped (immature): little or no spermatogenic activity in germinal epithelium; immature states of spermatogenesis (largely spermatocytes); no spermatozoa observed.	Undeveloped: pre-vitellogenic oocytes observed exclusively; oocyte diameter <250µm: cytoplasm stains basophilic with H&E.
1	Early spermatogenic: mostly thin germinal epithelium with scattered spermatogenic activity; spermatocytes to spermatids predominate; few spermatozoa observed.	Early development: >90% of oocytes pre-vitellogenic, remaining oocytes early to mid-vitellogenic; oocytes slightly larger (up to 300µm); late perinucleolus through cortical alveolar stages.
2	Mid-spermatogenic: germinal epithelia of moderate thickness; moderate proliferation and maturation of spermatozoa and equal mix of spermatocytes, spermatids and spermatozoa present	Mid-development: majority of observed follicles early and mid-vitellogenic; oocytes larger 300-600µm diameter, and containing peripheral yolk vesicles; globular and uniformly thick chorion (5-10µm in black basses, 10-20µm in common carp); cytoplasm basophilic, yolk globules eosinophilic.
3	Late spermatogenic: thick germinal epithelium; diffuse regions of proliferation and maturation of spermatozoa; all stages of development represented, spermatozoa predominate.	Late development: majority of developing follicles late vitellogenic; oocyte diameter 600-1000µm, eosinophilic yolk globules distributed throughout the cytoplasm; chorion thickness 10-30µm in black basses, 40-50µm in common carp.
4	N/A	Late development/hydrated: majority of developing follicles late vitellogenic; follicles much larger (>1000µm).
5	N/A	Post ovulatory: spent follicles, remnants of the theca externa and granulosa present.

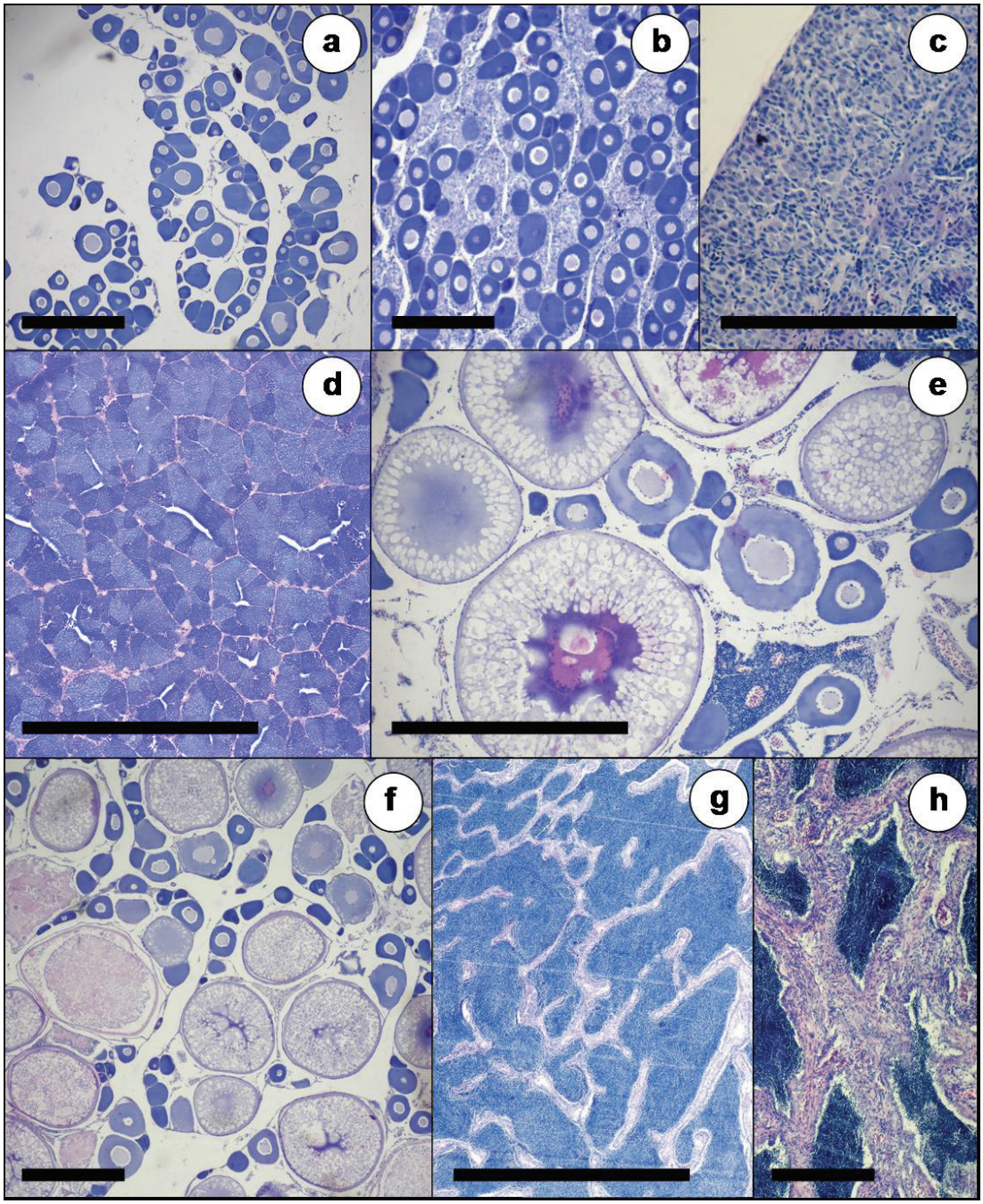

Figure 5: Immature and mature gonads of *Labeobarbus aeneus* from the Vaal River, South Africa, a, b) immature ovary with pre-vitellogenic follicles; c, d) immature testes, no spermatozoa; e, f) mature ovary, follicles in late-vitellogenic phase; g, h) mature testes; lobules filled with spermatozoa. Scale bars = 500µm.

Ageing using scales

The third and fourth lateral line scales were removed from 119 fish (Figure 3.3a) cleaned and then dried between two clean microscope slides (Figure 3.3b). Fish age estimates were obtained from the scales using a Nikon Profile Projector model 6CT2 at 20x magnification

and a 30 cm diameter viewing screen. Rings were counted along the scale surface from the nucleus towards the outer edge. Only those rings that were continuous throughout the scale were considered to be growth zones. Three replicate relative age readings were taken and noted. Readings were repeated after seven days. Replicate readings were taken so as to work out indices of bias and precision (Campana 2001) by a single reader, but between-reader analysis were not undertaken.

Ageing using otoliths

Left and right asteriscus otoliths were removed, cleaned, air dried and stored in 25 ml McCartney bottles from 167 fish (Figure 6). A valid growth zone was considered to comprise an alternating pair of opaque and hyaline zones (Figure 7b, c&d) that was continuous right round the otolith. Rings that were not continuous, and which lay between two continuous rings, were considered as false rings. Since otolith bands are formed over a period of time, the outer edge was not accepted as a growth zone (Brouwer and Griffiths, 2004). The fishes head was removed from the body (Figure 6a) to expose the gills and tissue surrounding the cranium (Figure 6b), this tissue was in turn dissected away to expose the cranium (Figure 6c), the otoliths were removed from auditory capsule after “cracking” the cranium (Figure 6d). Only whole otoliths were read (Figure 7a-d) as is the preferred way of ageing smallmouth yellowfish sectioning has been shown to not make rings more visible and (Weyl et al., 2009). Whole otoliths were interpreted using a Zeiss SC-40 stereo microscope under transmitted light (Figure 7e&f). The otoliths were immersed in methyl salicylate to make the rings more visible (Weyl et al., 2009). Three replicate readings were taken for the age determination and were done seven days apart in order to calculate indices of bias and precision (Campana, 2001) by a single reader and in addition a between reader analysis. To achieve between reader parity, two different readers each read the set of otoliths before comparing their results with each other. Indices computed from the data collected using the various methods was compared in order to identify the most appropriate ageing method.

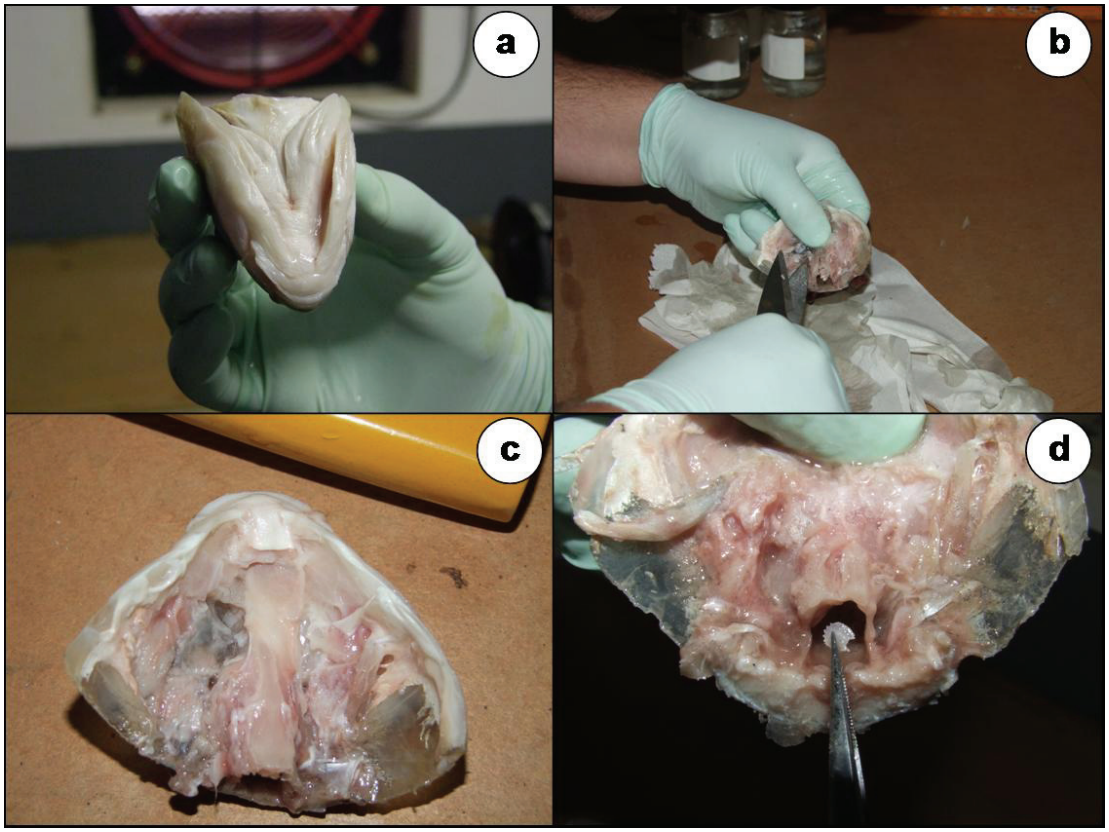

Figure 6: Photographs of the otolith removal process in *Labeobarbus aeneus*, a) fish head removed from body, b) gills and tissue being removed from around cranium, c) cleaned cranium before "cracking" the cranium, d) otolith removal from auditory capsule after "cracking".

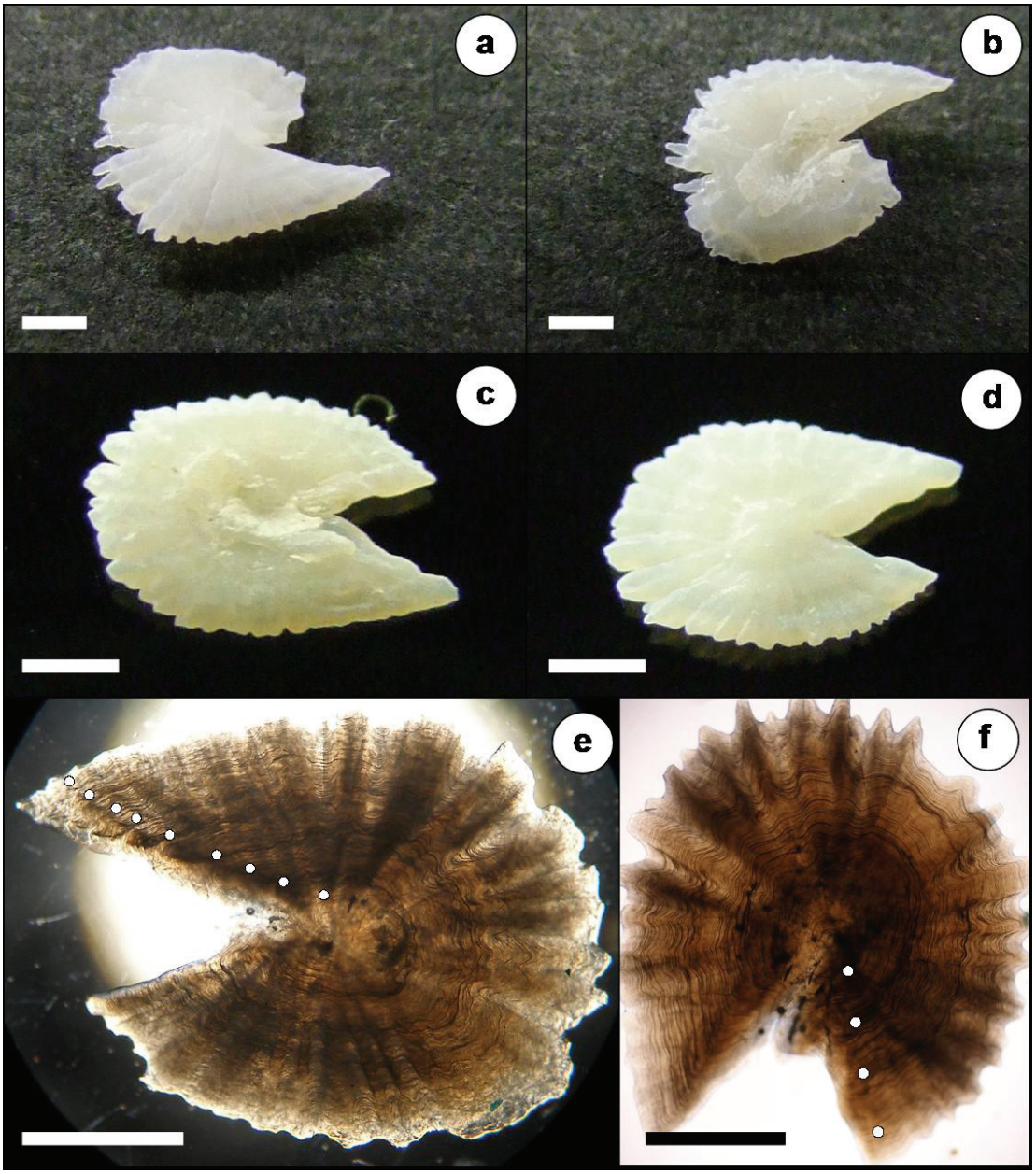

Figure 7: Photographs of distal (a;d) and proximal (b;c) views of whole otoliths, and micrographs (e;f) of whole asteriscus otoliths removed from *Labeobarbus aeneus* in the Vaal River, South Africa. Dots indicate growth zones. Scale bars = 1 mm.

Data analysis

Indices of bias and precision were calculated from three replicate relative age estimates obtained for each specimen and were compared for the three different methods of ageing (scales, whole embedded otoliths and sectioned otoliths). The precision of replicate age estimates was assessed using the average percentage error statistic (APE) proposed by Beamish and Fournier (1981) as well as the coefficient of variance (CV). Campana (2001) suggested that CV was a more robust method of determining precision than APE, but the

latter is included here for comparative purposes, because it has been more widely used (Campana, 2001). Bias was assessed using residual plots. For each of the three methods the residual for each reading of each specimen was calculated as the difference between the reading and the average of all three readings. The mean residual for each reading session was then calculated over all fish and plotted for each successive reading. No otolith readings were rejected in this study, even when readings did not coincide, i.e. where the three replicate readings differed by more than one or two. A single relative age estimate for each fish using each method was derived as the mode of the three replicate readings, assuming that the most frequently-occurring age estimate were most likely to be correct. Where a mode could not be calculated, e.g. because all three estimates were different, the mean of the three estimates rounded to the closest year was employed as a proxy. The 3-parameter von Bertalanffy growth model was fitted to the observed length-at-age data using a random effects modelling framework proposed by Cope and Punt (2007), error estimates for the growth curves were also generated from this model. Similarly growth curves were fitted to sexes separately. Between reader analysis was similar to the between structure analysis.

3 RESULTS

The results of the two main components of the study namely the sub-lethal physiological effects of yellowfish to angling stress and the complementary biological components including ageing, sexual maturity and growth of yellowfish are presented below.

3.1 Sub-lethal physiological effects

Water temperature

Water temperature observed in the study ranged from 11°C to 27°C depending on the time of year in which sampling took place. Mean total length (TL) and body mass of these fish were 504 ± 88 mm (217-666 mm) and 1.47 ± 0.68 kg (0.12-3.18 kg). Mean total angling time was 2 min 40 s ± 1 min 9 s (50 s-7 min 27 s) with a mean landing time of 1 min 30 s ± 55 s (20 s-5 min 56 s). A weak positive correlation was found between body mass and landing time (R^2=0.2248) (Figure 8). Following 72 hours in an aquarium, the mean plasma lactate concentration for the reference fish was 4.68 ± 1.67 mMol.L^{-1} (2.07-7.93 mMol.L^{-1}). Blood lactate concentrations in this group were significantly higher following hook and line capture than the concentrations found in the same fish following 72 hours in an aquarium ($P<0.05$);

however glucose and cortisol levels from reference fish were not significantly different to the post capture levels.

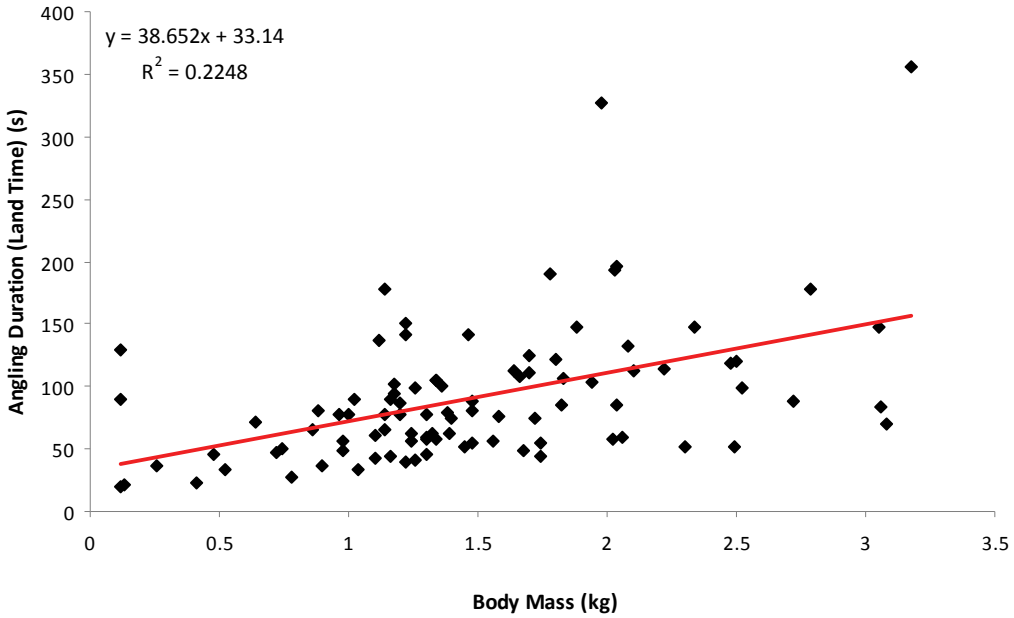

Figure 8: The relationship between smallmouth yellowfish body mass and landing time (n=96; P<0.05).

In consideration of the influence of water temperature as a variable on the data from individual yellowfish used in this study, individuals were grouped into four groups based on the temperatures they were caught in, namely those caught in 11°C, 19°C, 22°C and 27°C in groups 1-4, respectively. A one way ANOVA revealed that the landing time of the fish captured at the different water temperatures were not significantly different to each other (F=1.736, P=0.165). As the landing times of these groups were not significantly different from each other, the different blood parameters of each group were compared with one another. The one-way ANOVA analysis further revealed that plasma glucose and plasma lactate levels were significantly different between groups (Glucose – F=7.985, P<0.000; Lactate – F=7.776, P=0.0001) when calculated using water temperature. Only the plasma cortisol levels obtained between groups were not significant (F=1.419, P=0.243). The LSD *post-hoc* analysis showed that mean glucose levels at 11°C and 27°C were not significantly different (P=0.99), however they were significantly lower than the intermediate water temperatures of 19°C and 22°C (P<0.05), although the intermediate temperatures were also not significantly different to each other (P=0.99, Figure 9). While mean cortisol levels generally increased with water temperature, none of the groups were significantly different to each other (Figure 10). Mean post-capture plasma lactate concentrations also increased

with increasing water temperature, the LSD post-hoc analysis revealed that the 27°C temperature group was significantly higher to all other water temperature groups (P<0.05) (Figure 11).

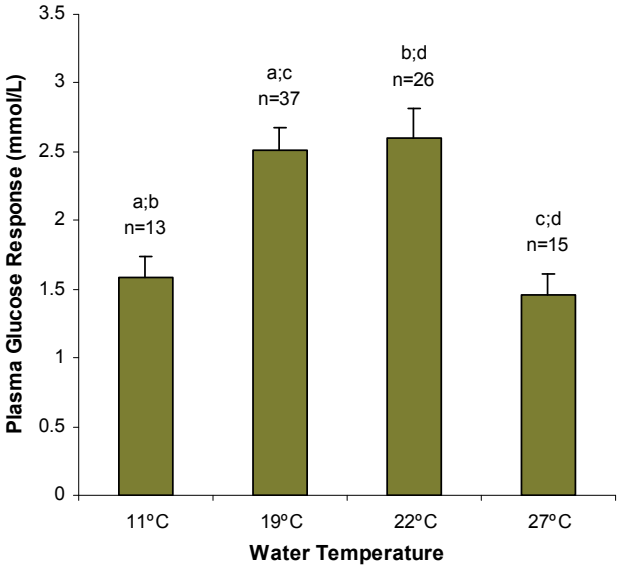

Figure 9: Plasma glucose response of smallmouth yellowfish angled during different water temperatures. Values are presented as mean ± SE; n=91. Means with common subscript differ significantly (P<0.05).

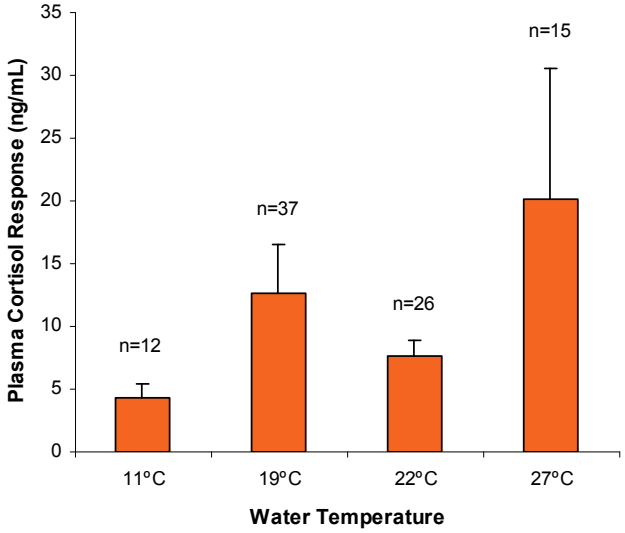

Figure 10: Plasma cortisol response of smallmouth yellowfish angled during different water temperatures. Values are presented as mean ± SE; n=96. Cortisol concentrations were not significantly different at varying water temperatures (P>0.05).

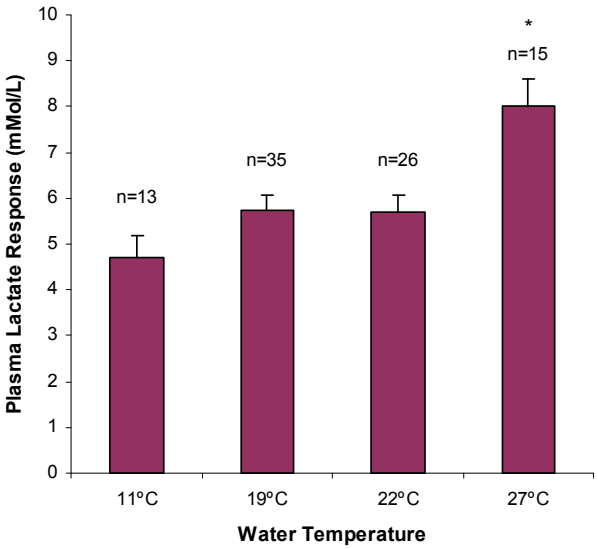

Figure 11: Plasma lactate response of smallmouth yellowfish angled during different water temperatures. Values are presented as mean ± SD n=87, * denotes that blood lactate concentrations were significantly greater than the other temperature groups ($P<0.05$).

Landing Time

In order to consider landing time, data were grouped into five groups based on the time taken to land fish including the reference group (group 1), less than one minute (group 2), one to two minutes (group 3), two to three minutes (group 4) and more than three minutes (group 5). Initially a one way ANOVA revealed that plasma glucose and plasma cortisol concentrations were not significantly different between groups when calculated using land time (glucose – F=1.535, P=0.198; cortisol – F=0.471, P=0.757). Mean plasma glucose for the different time intervals were however found to be negatively correlated to land time (R^2=0.9106); the longer fish were angled the lower the plasma glucose concentration (Figure 12), whilst plasma cortisol concentrations did not show any correlation (r^2=0.0043) (Figure 13). Although a few individuals (n=12) did show significantly (P<0.05) elevated plasma cortisol levels ranging from 18-160 ng.mL^{-1}, the remaining cohort had mean cortisol levels of 4.83 ± 3.94 ng.mL^{-1}. Plasma lactate concentrations were found to be significantly different when grouped by land time (F=4.005, P=0.005) (Figure 14), and mean lactate concentrations for the various time intervals were found to have a strong positive correlation (r^2=0.9687). LSD post-hoc analyses showed no difference between plasma lactate concentrations in the reference group and rapid capture fish (< 1 min); the reference group however was significantly different to the following groups: 1-2 min (P<0.05); 2-3 min (P<0.05); and > 3 min (P<0.05). Glucose (r^2=0.0622), cortisol (r^2=0.0685) and lactate (r^2=0.0278) concentrations showed no correlation to body mass.

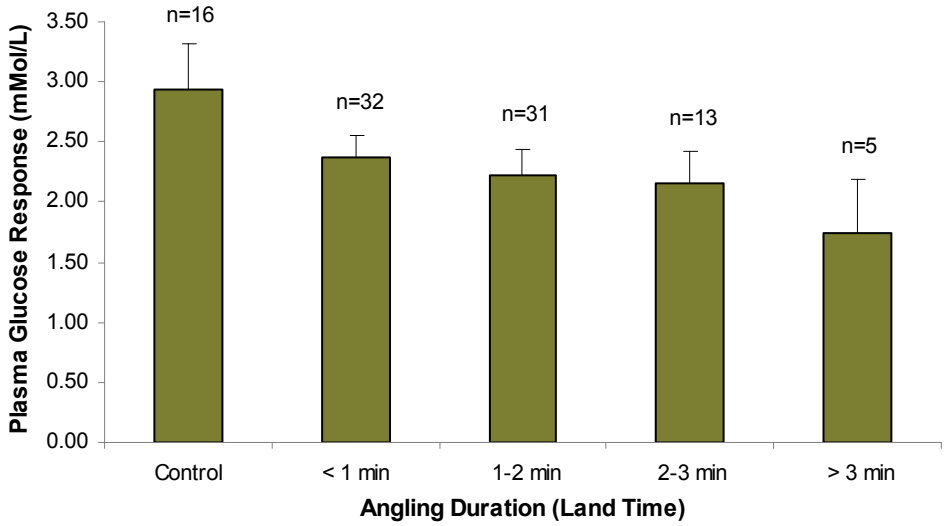

Figure 12: Plasma glucose response of reference and angled smallmouth yellowfish during different time intervals. Values are presented as mean ± SE; n=97.

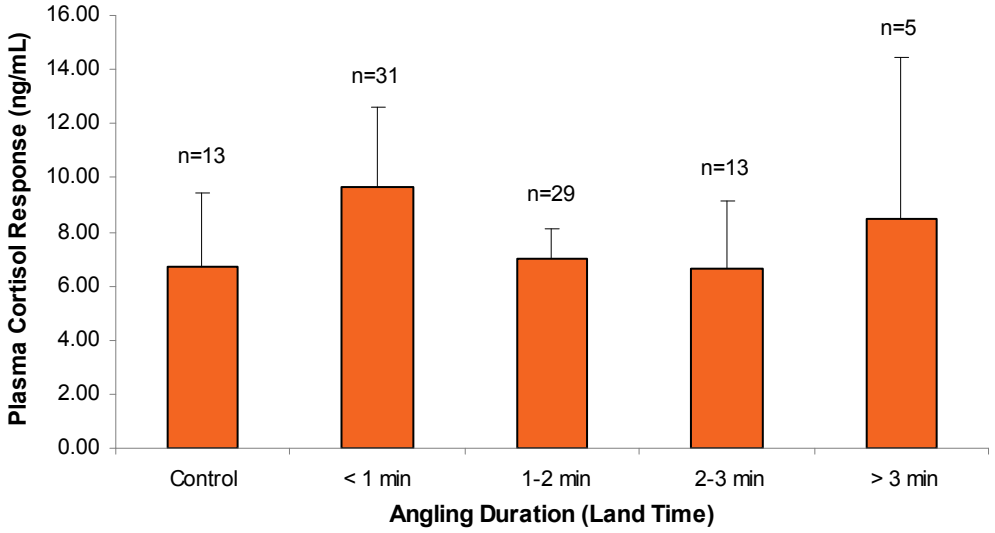

Figure 13: Plasma cortisol response of reference and angled smallmouth yellowfish during different time intervals. Values are presented as mean ± SE; n=91.

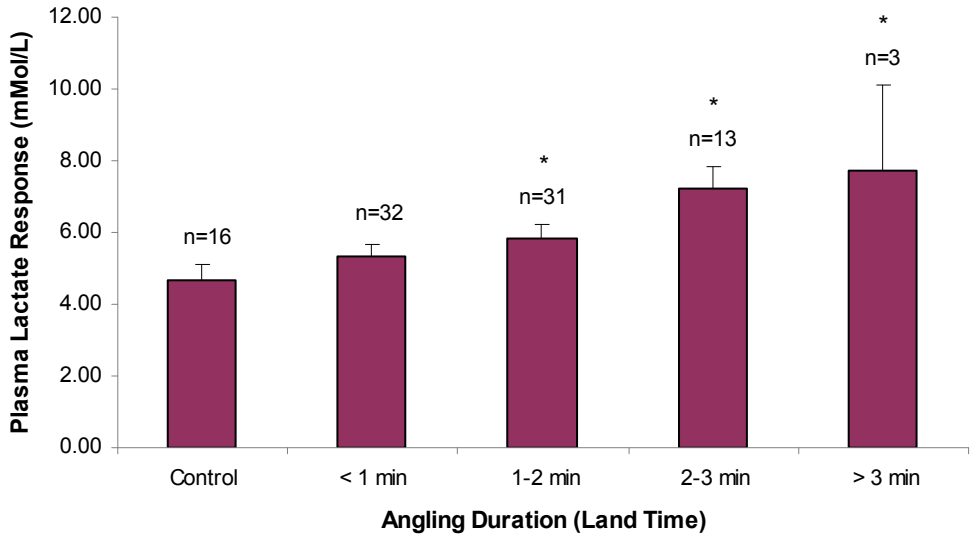

Figure 14: Blood lactate response of reference and angled smallmouth yellowfish during different time intervals. Values are presented as mean ± SE; n=95. * denotes that blood lactate concentrations were significantly greater than the reference group (P<0.05).

3.2 Ageing

Growth zone counts obtained from whole immersed otoliths showed the least variation among replicate readings, compared to those from scales (Figure 15). Most of the relative age estimates obtained from a given whole otolith were either identical or differed by only

one growth zone. In contrast, most age estimates obtained from scales differed by 2 to 3 growth zones, and sometimes by up to 6 or 7 growth zones.

When comparing age estimates from scales to those from whole immersed otoliths, it was found that scales showed greater variability by over-estimating and underestimating the age of fish in this study – much non-lineared bias (Figure 16).

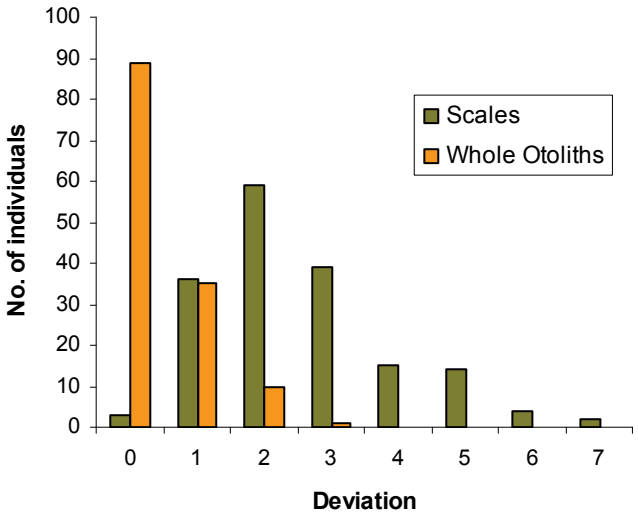

Figure 15: Deviations for the different ageing methods used. Deviations are the maximum difference between three replicate age estimates obtained from a given specimen.

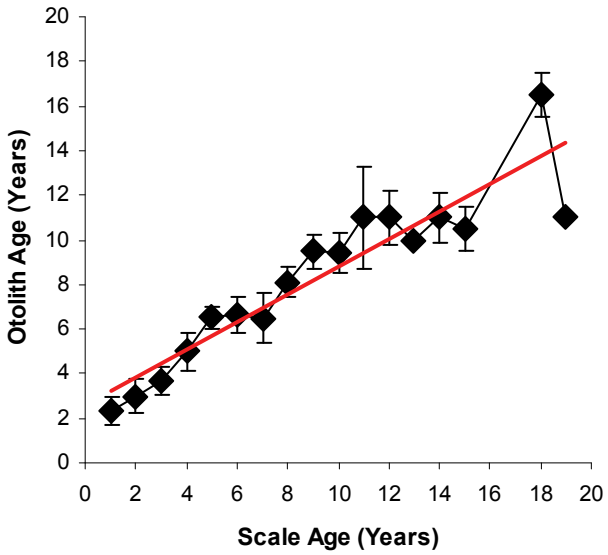

Figure 16: Plot comparing data obtained using the different ageing methods; age estimates obtained from the whole immersed otolith age vs. scale ageing method. Each error bar represents the 95%

confidence interval about the mean age assigned by using scales, for all fish assigned a given age using whole otoliths. Solid line indicates 1:1 relationship.

Some bias was apparent in the age data from scales (Figure 17a), with there being a tendency to count more growth zones at each successive reading whilst age data collected from the whole immersed otoliths showed very little if any bias (Figure 17b). In terms of precision, age data from whole otoliths were the most precise (Table 2), while age data from scales showed the lowest levels of precision.

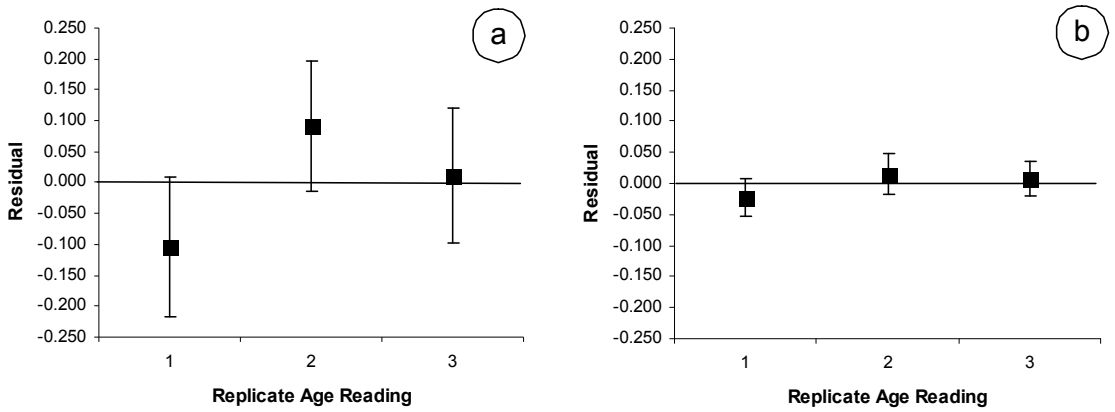

Figure 17: Plot illustrating mean residuals (± 1 SE) of each of 3 replicate age readings from the mean age obtained from (a) scales and (b) whole otoliths of *Labeobarbus aeneus*.

Table 2: Average percent error (APE) and coefficients of variation (CV) of replicate age readings recorded using different ageing methods (n = number of samples).

Ageing method	n	APE (%)	CV (%)
Scales	143	13.52	18.35
Whole immersed otoliths	167	2.57	3.39

The between reader analysis revealed that little age estimation bias was present (Figure 18) only in older individuals did the second reader count more growth zones than did the first reader, and in terms of precision the two readers were relatively precise (Table 3:).

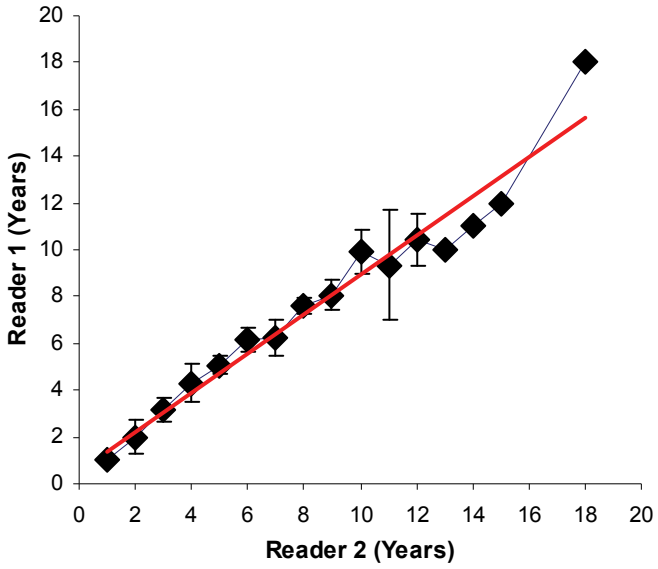

Figure 18: Plot comparing age data obtained from the different readers; age estimates were obtained from the whole immersed otolith ageing method. Each error bar represents the 95% confidence interval about the mean age assigned by one reader, for all fish assigned a given age by a second reader. Solid line indicates 1:1 correlation.

Table 3: Average percent error (APE) and coefficients of variation (CV) of age readings recorded using 2 different readers (n=number of samples).

Ageing method	n	APE (%)	CV (%)
Whole immersed otoliths	167	8.06	11.40

3.3 Sexual Maturity

Initial consideration of the length-weight relationships for male and female smallmouth yellowfish are described by the equations;

Female *L. aeneus*: Mass=*$FL^{3.2134}$, R^2=0.9424

Male *L. aeneus*: Mass=*$FL^{2.9189}$, R^2=0.9616

Male and female smallmouth yellowfish from the Vaal River differed very little in their length-to-weight ratio, females > 450 mm FL tending to be heavier than males. Females tended to double their mass from 410 mm FL (1 kg) to 500 mm FL (2 kg). Males sampled in this study seldom exceeded 2 kg and 600 mm FL, while females easily grew up to 3 kg and 600 mm TL.

The ratio of male smallmouth yellowfish to females caught was almost 1:1. Females measured between 300 and 600 mm FL (Figure 19) with very few being < 400 mm and the

largest individuals being > 550 mm. None of the males exceeded 600 mm FL and most were between 300 and 500 mm. Staging criteria of the gonad histology (Schmitt and Dethloff, 2000) showed that some male *L. aeneus* individuals matured at a minimum of 170 mm FL, but most matured at around 300 mm FL which corresponds to an age of four years, whilst females matured at a minimum of 350 mm FL, corresponding to an age of five to six years. Fifty percent maturity in males was reached at 289 mm FL in the length class 250 mm to 299 mm, and in females at 367 mm FL in the length class 350 mm to 399 mm (Figure 20 and Figure 21). All males in the 350 mm to 399 mm FL length class, and all females in the 500 mm to 550 mm FL length class were mature. Data from the whole otoliths (Table 3) fitted the von Bertalanffy growth curves well, with relatively few outliers and virtually no scatter (Figure 22a&b).

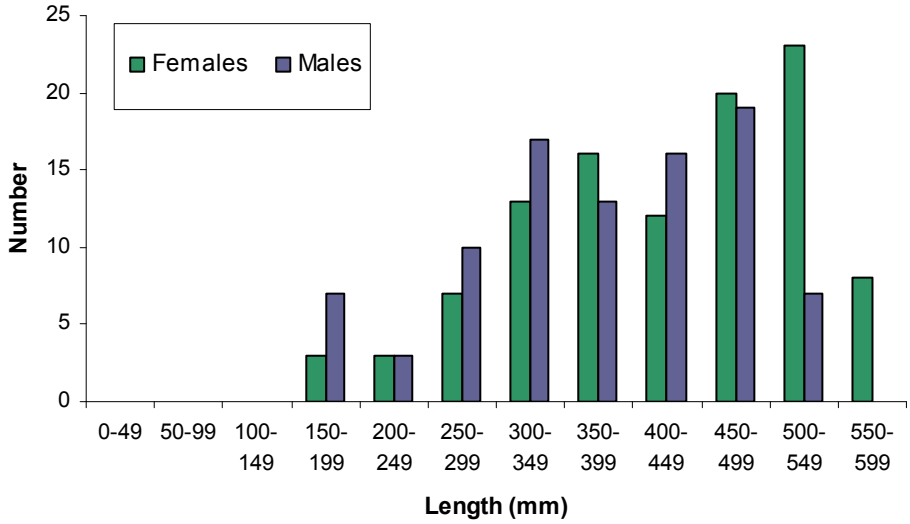

Figure 19: Length frequency analysis of both female and male *Labeobarbus aeneus.*

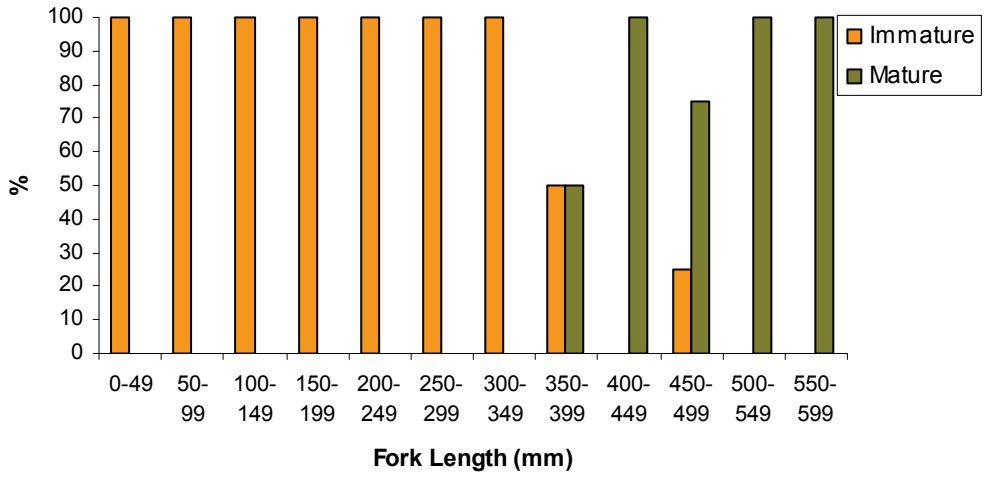

Figure 20: Bar graph showing relationship in percentage between immature and mature female *Labeobarbus aeneus* in each length class.

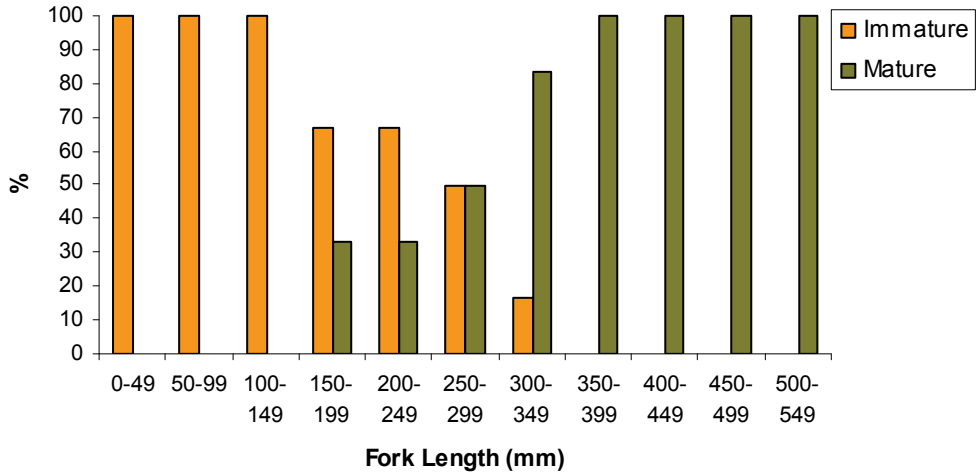

Figure 21: Bar graph showing relationship in percentage of immature and mature male *Labeobarbus aeneus* in each length class.

3.4 Growth

As mentioned earlier, assuming that a single growth zone is formed annually, males grow relatively faster for the first six years, reaching 350 mm FL, a length at which all were mature. Females also grow faster for their first six years of life to reach 400 mm FL, at which length all were mature. Males and females grew at a rate of 40-60 mm per year in their first six years of life. Males may only grow another 160-200 mm in length during their next 12 years of life, whereas females may grow another 200-250 mm in the same period. Both male and female growth rates slowed down after maturation and only reach asymptotic growth late in life, i.e. 8 or more years of age.

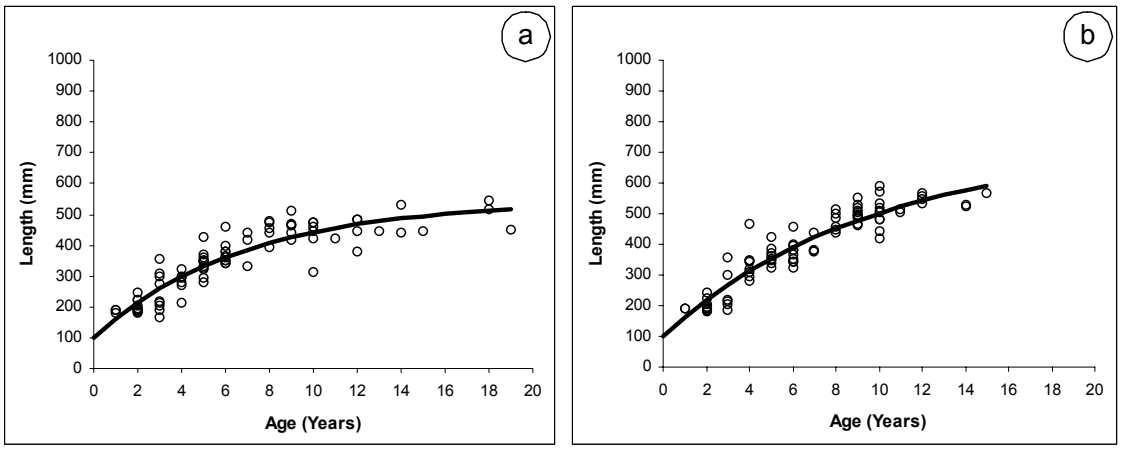

Figure 22: Size at age data obtained from analysis of whole otoliths of a) male and b) female *Labeobarbus aeneus.* The solid lines show the von Bertalanffy growth models fitted to the data (parameter estimates are provided in (Table 4).

Table 4: Parameters for the von Bertalanffy growth curves for each ageing method used (n = number of samples, error estimates are shown in parentheses).

Ageing Method	N	L-infinity	K	t_0
Whole otoliths	♀ = 83	541.72 (36.09)	0.149 (0.025)	-1.36 (0.33)
	♂ = 84	710.23 (79.87)	0.108 (0.025)	-1.38 (0.36)

Of the individuals considered in this study females grew larger than males reaching lengths in excess of 550 mm FL and weigh more than 3 kg, whilst the males collected were seldom over 500 mm FL and weighed slightly over 2 kg at most. Although they are smaller, males had a longer lifespan (19 years) than females (15 years) and thus grow slower than their female counterparts.

4 DISCUSSION

4.1 Sub-lethal physiological effects

Water Temperature

The water temperatures in this study ranged between 11°C and 27°C and represent the entire range of seasonal temperatures that these fish may be exposed to. Water temperature has been shown to inflict significant effects on post-capture levels of glucose, lactate and cortisol (Meka and McCormick, 2005). It has been suggested that temperature

has a significant influence on the physiological responses to exhaustive exercise – such as angling (Kieffer et al., 1994). In the current study, post-capture plasma glucose levels were shown to be lowest at the upper (27°C) and lower (11°C) temperatures (extremes), and highest at the intermediate temperatures of 19°C and 22°C respectively. Meka and McCormick (2005) found a significant correlation between glucose and temperature; in their study post capture glucose levels in rainbow trout were found to be increased with water temperature. The glucose concentrations shown in this study are non-indicative of the stress response, but may show the variation in the availability of energy during different times of the year and thus influenced by water temperature.

Post-capture plasma lactate levels matched the range of water temperatures sampled. Fish angled at the coldest (11°C) temperatures showed mean plasma lactate values similar to reference fish, whilst fish angled at intermediate (19°C and 22°C) temperatures showed mean lactate levels similar to fish angled for 2 minutes or less. Fish angled at the upper temperature extreme (27°C) showed mean plasma lactate levels similar to extended capture fish (i.e. fish angled for more than 2 minutes). Similarly rainbow trout (Meka and McCormick, 2005) and largemouth bass (Gustaveson et al., 1991) exhibited increased lactate levels as water temperature increased. Both these studies showed that at all temperatures, increased angling duration caused lactate levels to increase, but also that lactate concentrations were highest at the warmest water temperatures compared to the cooler water temperatures. The temperature a fish is acclimated to may influence the anaerobic energy production in fish (Kieffer et al., 1994). The lactate response found in fish from this study, showed that fish angled at lower and intermediate temperatures have the smallest response, whilst fish angled at the upper temperatures have the greatest response to angling. Kieffer et al. (1994) observed similar results, in that post exercise fish acclimated to higher temperatures (18°C) showed lactate concentrations two-fold greater than fish acclimated to lower temperatures (5°C). Therefore fish angled at higher water temperatures are put under greater stress and as a result under greater risk of a wide range of sublethal impacts and even mortality, compared to fish angled at lower water temperatures.

Angling periods

The blood parameters tested from angled Vaal River smallmouth yellowfish exhibit decreased glucose, increased cortisol (in some individuals) and increased plasma lactate concentrations with increased angling time. According to Booth et al. (1995b) the most physically demanding form of exercise stress in fish is capture by angling. Numerous studies

have shown that the longer fish are angled the greater the subsequent physiological response, (Pankhurst and Dedual, 1994; Thorstad et al., 2003; Meka and McCormick, 2005; Smit et al., 2009), which may result in elevated mortality rates (Meka and McCormick, 2005). Exhaustion can result from extended angling durations and is characterised by increased lactate levels, this is further magnified if the fish is exposed to air where the gill lamellae might collapse; consequently gas exchange is lost and CO_2 concentrations increase with a concomitant decrease in O_2 uptake (Casselman, 2005). Although all these factors might contribute to elevated levels of lactate, it is likely that the principal contributor to the physiological stress is the metabolic work done whilst hooked (Arlinghaus and Hallerman, 2007; Smit et al., 2009).

The plasma lactate response of *L. aeneus* to angling duration was significantly greater than references at every time interval, except at the 1 min time interval indicating that a large degree of metabolic stress is evident, even after short angling durations upwards of 1 min. Comparable results were shown by both Gustaveson et al. (1991) and Smit et al. (2009) in the response of largemouth bass and tigerfish, respectively. Tigerfish showed an immediate response with significantly higher blood lactate in fish angled for < 1 min and throughout the increasing time intervals when compared to references. Largemouth bass angled for 1 min or more also had a significantly higher blood lactate level than reference fish. Conversely, significant increases in lactate levels of angled rainbow trout were only found after 2-3 min from the start of angling (Meka and McCormick, 2005). Importantly Meka and McCormick (2005) used rapid angled fish (fish angled for < 2 min) as their reference fish (these fish will likely already have elevated lactate levels) and were fished at lower water temperatures (~10-13°C).

Keeping in mind the 16 reference fish that were kept successfully for 72 hr that had plasma lactate levels significantly lower than the post-capture levels (4.68 $mMol.L^{-1}$ vs. 5.74 $mMolL^{-1}$, respectively). Results show that *L. aeneus* plasma lactate levels were significantly elevated within 1-2 min (5.81 $mMol.L^{-1}$) after hook-up when compared to reference fish (4.68 $mMol.L^{-1}$). Smit et al. (2009) showed significantly elevated levels in less than 1 min (3.2 $mMol.L^{-1}$) after hooking when compared to reference fish (1.6 $mMol.L^{-1}$). Reference fish plasma lactate levels in this study are higher than in reference tigerfish (Smit et al., 2009). Meka and McCormick (2005) showed that rapid capture fish had lactate concentrations of ≤ 5 $mMol.L^{-1}$, which they suggested showed levels of free swimming fish (reference fish). The significant increase in plasma lactate levels within 1-2 minutes of angling indicates that there is a severe metabolic response, likely the result of increased muscular work, following hooking (Smit et al., 2009). The reference values for plasma lactate reported here are higher

than those reported for largemouth bass, 1.8 mMol.L^{-1} (Gustaveson et al., 1991) and Coho salmon, 1 mMol.L^{-1} (Milligan and McDonald, 1988).

The mean cortisol value of the angled *L. aeneus* was low and showed no increase with angling time. Post capture values were also not significantly different from the reference fish data, but were similar to rapid capture rainbow trout from the Alagnak River, which had cortisol concentrations of less than 11 ng.mL^{-1}; these values were considered to reflect those of free-swimming natural fish populations (Meka and McCormick, 2005). Consequently we propose that the cortisol values found during this study are also reflective of free-swimming "unstressed" fish. A few individuals (n=12) did however show significantly elevated cortisol concentrations of 18-160 ng.mL^{-1}. This might indicate that for fish with non-elevated levels, blood drawing may have been done before the cortisol response could be measured. Cortisol release from the inter-renal tissue is a delayed reaction to an induced stress because the response is dependent on various pathways; consequently, it is conceivable that it may take some time before the response can be seen in the blood (Barton, 2002; Iwama et al., 2004).

In this study glucose concentrations decreased as angling times increased in contrast to Silbergeld (1974) who found increased glucose concentrations in stressed fish. Although significant increases in plasma glucose have been observed within 5 minutes (Wydoski *et al*., 1976), numerous controlled studies have found that significant changes in glucose may take up to an hour to manifest (Ristori and Laurent, 1984; Carey and McCormick, 1998). The decrease in glucose with angling time was not significantly different to the levels of reference fish, Meka and McCormick (2005) similarly had no significant differences in glucose concentration with increased angling times. The lack of an increase in the glucose concentrations provides further support to the notion that the cortisol concentrations measured in this study are likely a reflection of resting values as greater circulating cortisol concentrations tend to result in higher glucose concentrations (Barton, 2002; Iwama *et al*., 2004).

Based on the data from the reference fish we are confident that lactate, cortisol and glucose levels in the reference group returned to concentrations that are analogous to the resting/free swimming levels of the population. Like Meka and McCormick (2005), we propose that the post capture lactate and cortisol levels found in this study represent the initial stages of a stress response caused by angling, and that the levels will continue to rise and will only peak some time after the initial stressor is applied (Barton, 2002; Iwama *et al*., 2004), but subside after 72 hours.

Body size

As the body mass of the smallmouth yellowfish caught increased, so did the angling duration (r^2=0.2248). This has been shown for numerous species in many different studies, namely; tigerfish (Smit et al., 2009), Atlantic salmon (Thorstad et al., 2003) and rainbow trout (Meka and McCormick, 2005). Glucose, cortisol and lactate levels showed no correlation to increased body mass. Smit et al. (2009) also found that lactate concentrations of reference fish data was independent of body mass and concluded that the metabolic stress caused by angling stress was the chief cause of elevated blood lactate levels (Meka and McCormick, 2005). Similarly we attribute decreased plasma glucose and increased plasma cortisol and lactate levels in this study to the unavoidable metabolic stresses of angling. The stress response in fish from this study has been shown to be independent of body size, but dependant of angling duration. Fish that were angled for longer periods were generally larger individuals, these increased angling times result in larger increases in lactate and decreases in glucose concentrations. This would suggest that the larger and oldest male and female individuals of the population would be placed under the greatest stress. These larger individuals would most likely be repeating spawners and deliver the largest contributions to the growth and replenishment of the population during spawning periods. The main season that *L. aeneus* are targeted by fly-fisherman is from mid-September to mid-April, these times correspond to their spawning season (Mulder, 1973; Tomasson et al., 1984; Weyl et al., 2009), which lasts from late-September through to mid-March, depending on the river water temperature and flow (Gaigher, 1976; Tomasson et al., 1984). Angling stress has been shown to affect spawning behaviour and spawning success in many species (Cooke et al., 2000) including largemouth bass and salmon, (Cooke et al., 2002, Thorstad et al., 2003). For *L. aeneus* the same might be true, but this requires further investigation.

Recovery, mortality and potential sub-lethal effects

Generally from the required time for post-capture plasma cortisol and glucose levels to return to resting levels is 24 hours following an intense stress event (Barton et al., 1986; Pickering and Pottinger, 1989; Pankhurst and Dedual, 1994; Iwama et al., 2004; Meka and McCormick, 2005; Arlinghaus and Hallerman, 2007). Post exercise lactate levels have been shown to clear relatively quickly, ranging from 8 to 18 hours, (Kieffer et al., 1994; Casselman, 2005). However, recovery times as well as the magnitude of the stress response were influenced by a number of factors; the severity and duration of the stress (Iwama et al., 2004), water temperature, recovery conditions and genetic variations between

populations (Pickering and Pottinger, 1989; Pankhurst and Dedual, 1994; Meka and McCormick, 2005). Therefore it makes the expectation tenable that fish exposed to extended capture durations as well as fish angled at higher water temperatures, would experience greater peak cortisol and lactate concentrations and thus require longer recovery periods than fish with shorter angling times and those fish angled at lower water temperatures.

Mortality from exhaustive exercise has been shown to manifest within several hours to several days post-capture (Dotson, 1982; Brobbel et al., 1996; Wilkie et al., 1996; Thorstad et al., 2003; Meka & McCormick, 2005). The exact cause of post-capture mortality is not known; it has been suggested that an intracellular acid-base disturbance induced partly through lactic acid production in the muscle may contribute for which plasma lactate is an indirect index of muscle lactic acid production (Wood et al., 1983). Increased plasma lactate levels have been shown to be associated with delayed mortality following exercise or hypoxia (Wood et al., 1983; Ferguson and Tufts, 1992). However, neither a threshold for plasma lactate nor other blood parameters have been established as a predictive mortality index (Meka and McCormick, 2005). Intracellular acidosis as a result of exhaustive anaerobic exercise as well as the collapse of the gill filaments due to air exposure may cause mortality due to the physiological disturbance and the associated energy depletion (Arlinghaus and Hallerman, 2007). Even slight increases of cortisol of 10 $ng.mL^{-1}$ have been shown to lead to the mortality of fish because the fish are left susceptible to various pathogens such as *Saprolegnia*, bacterial fin rot and furunculosis, all of which are well known as stress related diseases (Arlinghaus and Hallerman, 2007). Temperature has also been shown to affect mortality in fish species namely bluegill *Lepomis macrochirus*, Rafinesque, 1819 (Muoneke, 1992) and cutthroat trout (Dotson, 1982) where mortality rates increased up to 10% with increased water temperatures, and this was also shown for numerous bass species (Casselman, 2005). No mortality studies have been done on any African or South African species. Mortality caused by catch and release fishing is frequently under-estimated by both the anglers as well as the fisheries managers. After reviewing 118 catch and release studies, Casselman (2005) concluded that the average mortality arising from angling is around 16.2%, and therefore many anglers assume they are doing the right thing, and are not having an effect on the population; in reality many may die following release and have a marked impact on the population (Casselman, 2005). In the Vaal River although yellowfish dependent catch and release angling activities are well established (Brand et al., 2009) no associated increase in mortalities of yellowfish have occurred that can be linked to this activity. Apart from the maintenance of an industry with large economic benefits, yellowfish angling has extensive ecological and social benefits including awareness

of use and abuse of the goods and services of the Vaal River and the establishment of conservation initiatives for the Vaal River. As a result the advantages of the activity far outweigh the disadvantages that may include the mortality of up to 16.2% of the captured individuals. In order to limit the risk of these mortalities occurring some simple tips to ensure that catch and release practices remain sustainable have been established for yellowfish in the Vaal River (Appendix 1).

The increased plasma cortisol and lactate levels shown in this study are indicative of the exercise and the eventual stress responses that may have generated certain post-capture sub-lethal responses in individual *L. aeneus*. Many studies have demonstrated that fish may stop feeding following a stress event such as angling and this can result in decreased growth (Pickering et al., 1982; McCormick et al., 1998). According to Gregory and Wood (1999) this cessation of feeding and resultant decreased growth may be due to the effects of cortisol. In addition, other behavioural changes caused by the stress response may include avoiding predators, capturing prey (larger individuals of *L. aeneus*), as well as migration and habitat preference (rapids, riffles or deeper pools) (Iwama et al., 2004). Behavioural changes may reflect adverse changes as to how an animal perceives and responds to its environment (Iwama et al., 2004). The reproductive capacity of an individual or heavily fished population may decrease as energy used for such necessary life processes may be diverted in order to cope with the increased energy demand due to the stressor and as such decrease recruitment and productivity (Barton, 2002). It has been shown that increased cortisol levels while being of adaptive value to fish paradoxically also suppresses the immune response of the fish, thus leaving fish susceptible to disease during the recovery period (Arlinghaus and Hallerman, 2007). Although the effects of air exposure have not been assessed during this study, it is important to note the important role it may play in the stress response of angled fish. According to Thorstad et al. (2003), handling induces primary, secondary and tertiary stress responses. Handling duration has been shown to negatively affect angled fish even further (Meka and McCormick, 2005), by impairing ventilation and resulting in further muscular exertion (Bracewell et al., 2004; Arlinghaus and Hallerman, 2007). Mortality has also been shown to increase with air exposure (Arlinghaus and Hallerman, 2007). While out of water the gill lamellae collapse and inhibit gas exchange, further affecting acid-base concentrations (Ferguson and Tufts, 1992) and can lead to substantial physiological disturbances and result in longer recovery times (Arlinghaus and Hallerman, 2007). In a study on pikeperch Arlinghaus and Hallerman (2007) found that fish with no air exposure had the lowest mortality rates, whilst fish that were exposed to air showed no significant difference between shorter and longer duration air exposure times – thus even short durations of air exposure can result in increased mortality rates.

4.2 Length-weight relationships

The length-weight relationships of both sexes have the constant (n) close to 3 which is accepted as a general trend in fishery biology, due to a specimens' mass changing at the cube of its length, provided the parameters of shape and specific gravity remain the same (Carlander, 1969). Both relationships also have high R^2 values, which show that the length-mass relationships for each of the sexes are very well correlated, due to the fact that there are very few outliers for both sexes and these are probably due to them either being in a better or a worse condition or in varying stages of gonadal development. Females sampled in this study grew larger than the males, this is similar to other studies pertaining to *L. aeneus* (Ellender, 2008; Weyl et al., 2009). This phenomenon has been reported for several other African freshwater species, for example; *Hydrocynus vittatus* (Gerber et al., 2009), *Schilbe intermedius* (Merron and Mann, 1995) and *Hepsetus odoe* (Merron et al., 1990).

4.3 Gonads and maturity

Fishes examined by Jones and Hynes (1950) were considered to be mature when testes showed active cell division, and ovaries showed well developed oocytes whilst immature gonads had no signs of spermatogenesis or oogenesis for testes and ovaries respectively. In this study a 0 to 5 gonadal staging index was used. Merron and Mann (1995) similarly used a 1 to 6 gonadal maturation index (G.M.I.) to determine if specimens of *Schilbe intermedius* from the Okavango Delta, Botswana, were sexually mature.

Labeobarbus aeneus requires very specific conditions for successful spawning and reproductive activity is triggered primarily by temperature in perennial rivers and by a combination of temperature and increased flow rate in seasonal rivers (Gaigher, 1976; Tómasson et al., 1984). The spawning season for *L. aeneus* is in summer from October to January, depending on locality (Mulder, 1973; Stadtlander, 2007; Tómasson et al., 1984). Spawning appears to be controlled primarily by temperature (Tómasson et al., 1984). *Labeobarbus aeneus* has a minimum temperature requirement of 18.5°C to initiate spawning and breeds in flowing water and they therefore must ascend the rivers to spawn in gravel beds (Jubb, 1972b; Mulder, 1973; Tómasson et al., 1984; Cambray et al., 1986). Eggs are laid on gravel beds, where the eggs then fall in-between the interspersed gaps of the stones and gravel. Here they will be agitated and constantly supplied with oxygen due to river flow (Jubb, 1972b). Courtship behaviour has been observed for *L. aeneus,* where males chase the females (Stadtlander, 2007), but it is suspected that spawning occurs before sunrise or just after sunrise (De Villiers and Ellery 2008). *Labeobarbus aeneus* individuals are also considered to have two spawning runs per season (Stadtlander, 2007). A 50 cm female may carry up to 60 000 eggs (Tómasson et al., 1984). While they may carry

these many eggs, Mulder and Franke (1973) found that *L. aeneus* females ranging from 42 to 55 cm would produce only between 29 000 and 41 000 eggs. Egg development is temperature dependent and takes about 5 days at temperatures of 20-22°C to hatch and the larvae are initially immobile with large yolk sacs and commence swimming approximately 6 days after hatching (Tómmasson et al., 1984).

Males mature at a younger age and a smaller size than the females. When comparing data collected from different localities (see Table 5.4) it appears that age and length at maturity for *L. aeneus* is locality specific (Mulder, 1973; Tómasson et al., 1984). Fish from the Orange and Great Fish River generally mature at lengths exceeding 300 mm FL. Vaal River fish in this study matured later at ages of 4 years (male) and 5 years (females), similar ages and lengths to those found by Mulder (1973) also from the Vaal River (Table 2). The difference in length and age at maturity between this Vaal River study and other populations can be attributed to the fact that *L. aeneus* is phenotypically plastic (Tómasson et al., 1984; Ellender, 2008), thus their age at maturity is influenced by the environmental conditions and recruitment success of previous spawning runs (Ellender, 2008). Different ageing techniques may also contribute to the differences in age-at-maturity.

Ageing methods and consistency of age determinations

Previous studies have shown that whole immersed asterisci otoliths are the preferred method of ageing populations of *L. aeneus* (Ellender 2008; Weyl et al., 2009). Similarly in this study whole immersed asterisci were found to be the preferred method to age *L. aeneus* from the Vaal River, as results from these otoliths showed the least bias and were found to be several times more precise than scales. Whole otolith APE and CV values (2.57% and 3.39% respectively) were the closest to “acceptable” APE and CV values (5.5% and 7.6%, respectively) described by Campana (2001), when compared to scales (13.5% and 18.4%, respectively).

Differences in the age estimations between readers as well as methods are determined by replicate age determinations of a sample of fish. Subjectivity during the process of determining fish age contributes varying degrees of error to age determinations. Ageing error can affect the precision of replicate age readings, i.e. the reproducibility of readings on a particular structure. Measures of precision may be falsely magnified by any bias which may exist between readers or readings by a single reader. Values of precision are relative and as such no single value can be described as acceptable for all of the fish species, so

per se the APE and CV values listed for the between reader analysis in this study may be considered to be “acceptable” (Campana et al., 1995).

In a similar study it was found that more growth zones may be visible on sectioned tigerfish otoliths than on the surfaces of the whole otoliths (Gerber et al., 2009), because of growth increments not forming equally on all parts of the otolith, and annuli of older fish crowding at the edges (Dwyer et al., 2003). These whole tigerfish otoliths underestimated the relative age of fish due to the “stacking” phenomenon of the growth zones. Thus newly-formed growth zones would consequently not be visible by observation (Gerber et al., 2009). Smallmouth yellowfish asteriscus otoliths do not grow thicker as it widens and the growth zones are deposited in a concentric fashion and no “stacking” takes place. Unlike whole tigerfish otoliths, whole smallmouth yellowfish asteriscus otoliths can therefore be successfully used for determining growth increments.

4.4 Age and Growth

In consideration of the longevity of other populations of *L. aeneus* namely, Glen Melville Reservoir (8 years longevity), Great Fish River (10 years longevity) and Lake Gariep (12 years longevity), the Vaal River *L. aeneus* population are much longer lived (males 19 and females 15 years). The largest individuals aged in this study were only slightly over 3 kg, and individuals of more than 7 kg have been reported (Skelton, 2001), indicating that Vaal River *L. aeneus* potentially has a greater longevity than recorded here. Reasons for the difference in the maximum age from the various populations may be that the fish from the Great Fish River do not grow as old as the Vaal River fish since they did not naturally occur in that region, but were introduced via the Fish River tunnel that transports water from the Orange River to the Great Fish River. This might also be true for the fish in Lake Gariep and Xonxa Dam (Richardson et al., 2009) as a lake is not the natural habitat of smallmouth yellowfish. However, further research is needed to determine the specific reasons that might affect the maximum age difference between Vaal River, Lake Gariep, Xonxa Dam and Great Fish River smallmouth yellowfish. There are few otolith age data for African freshwater fish species available; however Vaal River *L. aeneus* has a similar average lifespan (20 years) to their cyprinid counterpart the Vaal-Orange largemouth yellowfish *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913) (17 years) from Lake Gariep (Ellender, 2008). *Labeobarbus aeneus* also has a longevity similar to that of other African species namely: *Hydrocynus vittatus* from the Okavango Delta (Gerber et al., 2009), *Clarias gariepinus* from Glen Melville Reservoir and red breasted tilapia, *Tilapia rendalli* (Boulenger, 1897) from Lake Chicamba who have individuals reaching relative ages of 20+ years and

validated ages of 15+ years (Weyl and Booth, 2008) and 16 years (Weyl and Hecht, 1998), respectively.

Other African species which have been aged, but only have a lifespan of ⅓ to ½ of that found for *L. aeneus* in this study include: the cyprinid, redeye labeo, *Labeo cylindricus* (4 years) from Lake Chicamba (Weyl and Booth, 1999); the cichlids *O. macrochir* (11 years) (Booth and Merron, 1996), *O. andersonii* (13 years) (Booth et al., 1995a) from the Okavango Delta, as well as Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852) (10 years) from Lake Chicamba (Weyl and Hecht, 1998); and lastly the mormyrid, bulldog, *Marcusenius pongolensis* Peters, 1852 (7 years) from Mnjoli Dam, Swaziland (Khumalo, 2006).

Table 5.4: Von Bertalanffy growth parameters of South African Smallmouth yellowfish (*L. aeneus*) populations; summarised and adapted from Ellender, 2008 and Weyl et al., 2009. $\mathbf{L_{mat}}$ – length at maturity; $\mathbf{A_{mat}}$ – age at maturity; **L-infinity** – asymptotic length; **K** – Brody growth coefficient; $\mathbf{t_0}$ – age at 0 length. ([1]Koch, 1975; [2]Hamman, 1981; [3]Ellender, 2008; [4]Weyl et al., 2009; [5]Tòmasson, 1983; [6]Mulder, 1973; [7]Current study).

	Maturity		***VBGF Parameters***		
Location	$\mathbf{L_{mat}}$	$\mathbf{A_{mat}}$	**L-infinity**	**K**	$\mathbf{t_0}$
Males					
Boskop Reservoir[1]	-	-	345	0.195	-0.07
Lake Gariep[2]	210	3	676	0.110	-0.09
Lake Gariep[3]	231	3	398	0.330	-0.34
Glen Melville reservoir[4]	297	4	407	0.193	-0.20
Great Fish River[4]	247	3	374	0.403	-0.06
Lake van der Kloof[5]	-	3	603	0.190	0.52
Vaal River[6]	280	4	1115	0.059	-0.48
Vaal River[7]	289	4	542	0.149	-1.36
Females					
Boskop Reservoir[1]	-	-	1560	0.031	-0.53
Lake Gariep[2]	310	5	684	0.120	-0.20
Lake Gariep[3]	354	5	491	0.236	-0.29
Glen Melville reservoir[4]	327	6	13259	0.001	-6.85
Great Fish River[4]	333	4	516	0.235	-0.15
Lake van der Kloof[5]	300	4	710	0.160	0.47
Vaal River[6]	340	5	1221	0.051	-0.51
Vaal River[7]	367	5	710	0.108	-1.38

Growth in *L. aeneus* from the Vaal River is generally fast up to the attainment of sexual maturity; thereafter, growth slows. Growth in fish from this study is slower than that described by Mulder (1973) who examined age in smallmouth yellowfish, in the Vaal River; this is likely attributed to the fact that Mulder (1973) used scales, which have been known to exaggerate growth in many fish species and are known to be unreliable (Booth et al., 1995a).

5 CONCLUSIONS

This specific study presents information regarding the physiological effects of C&R fishing on only the second southern African species. The results from *L. aeneus* differs to a certain

extent with that of *H. vittatus*, this confirms once again that the physiological response to angling is specific and it is important that research focus on all targeted species and populations. As such the results obtained in this study directly apply to *L. aeneus* in the Vaal River, in the vicinity of Potchefstroom alone, but may prove to be representative of other popular C&R populations of yellowfish throughout South Africa. Water temperature along with angling times were the influential factor in the range of glucose, cortisol and especially lactate levels in this study.

Whole immersed otoliths are the best method for ageing smallmouth yellowfish from the Vaal River and should be used as the preferred method for any future ageing studies. Scales obtained from the lateral line should be used with caution, as they can cause disagreement in fish ageing, and thus lead to incorrect fisheries management. More work needs to be done to validate the formation of the first otolith annulus by collecting and analysing otoliths from recruits at short intervals, and to validate the number of growth rings formed per year.

The original aims proposed to test the hypotheses established for this study were to carry out assessments of the effects that selective angling activities (C&R) may pose to populations of *Labeobarbus aeneus* from the Vaal River, South Africa, to determine age, growth and size at maturity for this population. These aims were all achieved and now by reviewing the results obtained from the different chapters we can now revisit the hypotheses in order to accept or reject them.

***Hypothesis 1*:** Blood plasma lactate, glucose and cortisol levels can be used as bioindicators of physiological stress in *L. aeneus*. Plasma lactate, glucose and cortisol have been shown to be useful bioindicators of stress in the species studied. Glucose and cortisol levels should however be viewed with caution as changes in levels of these two stress indicators may take some time to manifest. Changes in lactate could immediately be seen. This hypothesis is therefore partially accepted.

***Hypothesis 2*:** Larger fish experience longer angling durations and experience the greatest angling stress and therefore stand less chance of survival. Larger fish are indeed angled for longer periods of time, and these increased angling times result in a greater stress placed on these larger fish. This hypothesis is therefore accepted.

***Hypothesis 3*:** Increased angling duration results in a greater stress response, i.e. the longer the landing time and handling time the greater the stress response in *L. aeneus,* this

may be further exacerbated during spawning periods. An increased angling duration has been shown to elicit a greater stress response in individual fish, this stress response is not necessarily increased during spawning periods, but the negative impacts following this stress event may be greater than in other instances. The first section of this hypothesis is accepted, but the section pertaining to the spawning periods has not been tested sufficiently to make a conclusion.

Hypothesis 4**:** During extreme water temperatures the negative effects of angling become greater, i.e. increased water temperature results in a greater angling stress response. Fish were put under greatest stress when angled at the upper extreme temperatures (27ºC). This hypothesis is therefore accepted.

Hypothesis 5**:** *L. aeneus* are fast growing, fast maturing and able to reproduce from an early age. *L. aeneus* grows relatively fast and reaches maturity late in life (at a ⅓ to ½ of their lifespan). The first part of this hypothesis pertaining to fast growth is accepted, but the latter part regarding the maturity is rejected.

Hypothesis 6**:** Age data obtained from scales do not reflect the true age of fish. Scales sampled from *L. aeneus* have been shown to be unreliable and show the lowest levels of precision of the two ageing structures and methods. This hypothesis is therefore accepted.

This study shows that C&R results examining the physiological effects are significant. The measurable physiological stress manifests itself within a short period of time, i.e. < 2 min, following the onset of hooking. The negative impacts associated with an increase or decrease in the measured variables (plasma glucose, cortisol and lactate), have been described. The most important of these impacts is the potential for altered reproductive behaviour and reduced growth of the individual. Although no mortality has been shown in this study it may indeed be occurring. Mortalities can occur when following ineffective C&R methodologies, when angling at extreme water temperatures or when fish are angled for prolonged periods. Refer to the catch, revive and release guidelines (Appendix 1).

Should post release mortality of angled fish be occurring, this could be viewed in the same light as fish that are caught and kept for consumption for example. Both of these practices essentially eliminate the affected individuals from the population and in excess both practices may impact on the sustainability of the yellowfish populations. Furthermore, fishermen tend to target the larger individuals of a population, the larger individuals of the studied populations that are generally female. The removal of large females from the

population can have negative consequences as these individuals make up a large, important part of the broodstock of the river as they have higher fecundity, are able to spawn more regularly and potentially have a better egg quality and associated larval survival rates, relative to smaller individuals (Ellender, 2008).

The phenotypic plasticity of yellow fish populations should allow them to respond to fishing pressures and in so doing mitigate the risk of mortality within the population (Brouwer and Griffiths, 2005). If C&R activities occur in excess and result in increased adult mortality it would lead to a decrease in age-at-maturity of the population; this in turn would result in decreased growth rates because of this affect on energy being allotted to reproduction (Brouwer and Griffiths, 2005). No decrease in age-at-maturity was found in the population of *L. aeneus* sampled in this study, both the age-at-maturity and size-at-maturity are similar to what Mulder (1973) found. However, from the results of this study, there currently seems to be little to no direct effect on the populations considered. As the dramatic increase in the popularity of recreational sport fishing is relatively recent it is important that measures are put in place to prevent long term impacts of C&R angling to affect smallmouth yellowfish populations, as the real effect of C&R might not be post release mortality but rather in the altered reproductive behaviour of individuals within the population, thus affecting recruitment.

The life history traits of *L. aeneus*, including the extended time taken for yellowfish to reach maturity, make them vulnerable to exploitation. These species are relatively slow growing and long lived, reach maturity relatively late in life (⅓ to ½ of their lifespan) and have relatively high fecundities. According to King and McFarlane (2003) these characteristics classify these populations as intermediate strategists. Management recommendations for intermediate strategists are to maintain a critical spawning biomass (King and McFarlane, 2003). These aspects should be considered in order to establish a management strategy for the yellowfish populations in the Vaal River.

6 RECOMMENDATIONS

6.1 Recommendations to anglers for effective fly fishing C&R practices

Stress and mortality of fish can be decreased by following general catch and release guidelines (Casselman, 2005). Recommendations made here have been adapted from Casselman (2005), specifically for this study. These recommendations apply to both tigerfish and smallmouth yellowfish, except where species specific recommendations are made.

In terms of angling techniques appropriate gear should be used in order to minimise angling times and thus decrease physiological impacts (consider Appendix 1):

- When targeting smallmouth yellowfish a minimum of 5/6 weight fly rods should be used, as well as appropriate leaders and tippets (to prevent breaking).
- Barbless hooks are recommended because they are easier to remove and therefore decrease handling times as well as air exposure times.
- Avoid angling during extreme water temperatures, specifically the upper extremes (upwards of 25ºC), rather fish at intermediate temperatures in order to reduce the physiological stress.
- Fish exhaustion should be prevented by retrieving fish as quickly as possible, therefore angling times should be kept under three minutes for the species.

When landing and handling a fish the following should be considered:

- Fish should be landed by hand where possible, if a net is necessary a soft knotless rubber net should be used as these have been shown to have the least effect on fish when compared to other net types (Casselman, 2005).
- When large fish are being landed a fish cradle should be employed in the landing process (large fish are usually more difficult to land and can cause mechanical damage to themselves when jumping around).
- Minimise air exposure by keeping the fish in the water as much as possible, air exposure has been shown to already have a marked effect.
- Never place your fingers through the gills or in the eyes of the fish.
- The jaws and vertebrae of large fish may be damaged when holding them by the jaw. Hold large fish horizontally and support the body so as not to cause damage to internal organs. Do not hang large fish vertically from lip-grips for extended periods.
- Use wet hands or wet cloth gloves when handling a fish, to protect the fish's protective outer mucus layer.
- Preferably photograph the fish while it is in the water, if this cannot be done the camera should be ready prior to the fish being lifted out of the water so that air exposure times can be minimised.
- Remove the hook as quickly as possible, with the fish underwater, and where necessary use long nosed pliers and be sure to keep them at hand.

When reviving the fish it is important to keep the following in mind:

- If current is available keep the fish upright with its head facing into the current.
- When no current is available, the fish should be moved slowly back and forth till fish are able to swim away powerfully on their own accord.

- Let the fish swim away when it starts to show increased activity (struggle).

6.2 Management Recommendations

Minimum size limits should be based on age-at-maturity, this allows fish to spawn once or twice before entering the fishery (Ellender, 2008). *Labeobarbus aeneus* currently has a minimum size limit of 300 mm FL and bag limit of two fish (Ellender, 2008). The findings of this study suggest that the established limitations remain unchanged, however if fishing pressures were to increase these restrictions would have to be revised. Continual monitoring will have to be done in both fisheries to ascertain exploitation levels and to employ management strategies relevant to the dynamics of the fisheries.

Due to *L. aeneus* being targeted during their breeding period (mid September through March) and because they are even more easily targeted when forming spawning aggregations, a seasonal closure/s may be recommended. This closure should be during major spawning events which occur during the earlier parts of the spawning period. This seasonal closure may in turn also benefit the other species with similar spawning periods present within the system. A seasonal closure should however be considered as a 'last resort' and fishermen must rather be discouraged from targeting these spawning aggregations which form during the *L. aeneus* spawning period.

6.3 Future Research

Based on the identification of gaps in our knowledge concerning the studied species, *L. aeneus* during the course of this project the following future research is proposed.

- To validate the periodicity of growth increment deposition in the *L. aeneus* population studied. Validation methods include chemical marking by injecting specimens with oxytetracycline and marginal zone analysis.
- To determine the peak spawning period of *L. aeneus* in the Vaal River. Methods to be used include macroscopic and microscopic analysis of gonads together with the Gonado-somatic index (GSI).
- Establish levels of the tested parameters (plasma glucose, cortisol and lactate) that may cause mortality of the individual in the studied species.
- Telemetry tag angled fish to discover how the fish is affected and if and how its behaviour may change post-release as well as to determine if and when mortality occurs.

- Research concerning angling stress must be expanded to include more of the targeted species in southern Africa as well as include all the different angling methods used to target these species.
- Compare populations of *L. aeneus* (or any other studied species) that are subjected to either a C&R fishery or a consumptive fishery, to determine the effects that these different fisheries have on the population dynamics and ecology of these populations.

Natural freshwater resources are in decline due to decreased quantity and quality of our freshwater ecosystems and increased demands of a growing human population. Due to this increased reliance on our freshwater systems, increased pollution and the ever present threat of climate change, it is imperative that continual monitoring and research pertaining to these freshwater ecosystems takes place. Vitally, this will in turn provide ecosystem managers with the tools to adequately conserve these dynamic systems and the valuable natural resources harnessed within them.

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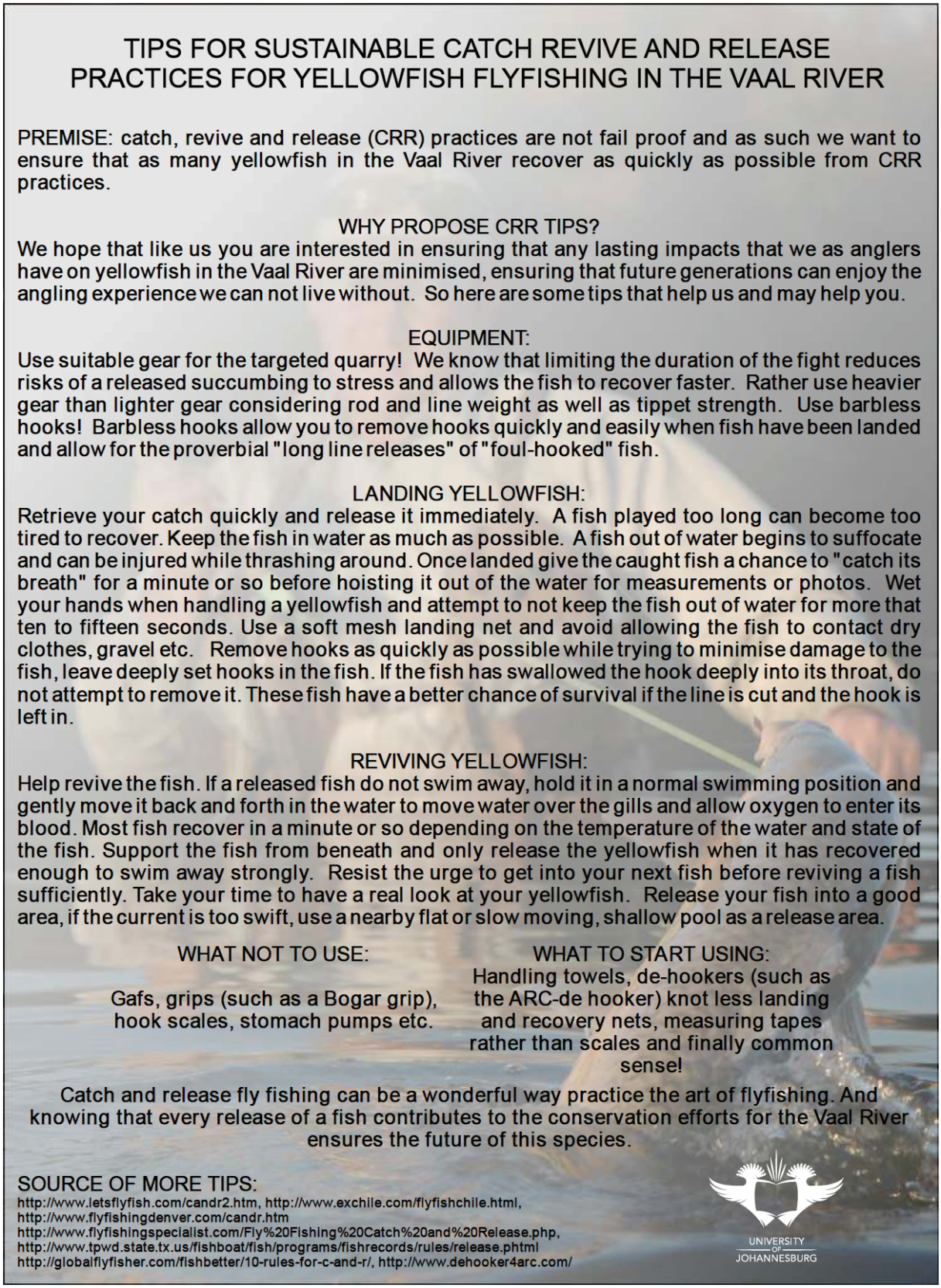
8 APPENDIX:



TIPS FOR SUSTAINABLE CATCH REVIVE AND RELEASE PRACTICES FOR YELLOWFISH FLYFISHING IN THE VAAL RIVER

PREMISE: catch, revive and release (CRR) practices are not fail proof and as such we want to ensure that as many yellowfish in the Vaal River recover as quickly as possible from CRR practices.

WHY PROPOSE CRR TIPS?

We hope that like us you are interested in ensuring that any lasting impacts that we as anglers have on yellowfish in the Vaal River are minimised, ensuring that future generations can enjoy the angling experience we can not live without. So here are some tips that help us and may help you.

EQUIPMENT:

Use suitable gear for the targeted quarry! We know that limiting the duration of the fight reduces risks of a released succumbing to stress and allows the fish to recover faster. Rather use heavier gear than lighter gear considering rod and line weight as well as tippet strength. Use barbless hooks! Barbless hooks allow you to remove hooks quickly and easily when fish have been landed and allow for the proverbial "long line releases" of "foul-hooked" fish.

LANDING YELLOWFISH:

Retrieve your catch quickly and release it immediately. A fish played too long can become too tired to recover. Keep the fish in water as much as possible. A fish out of water begins to suffocate and can be injured while thrashing around. Once landed give the caught fish a chance to "catch its breath" for a minute or so before hoisting it out of the water for measurements or photos. Wet your hands when handling a yellowfish and attempt to not keep the fish out of water for more that ten to fifteen seconds. Use a soft mesh landing net and avoid allowing the fish to contact dry clothes, gravel etc. Remove hooks as quickly as possible while trying to minimise damage to the fish, leave deeply set hooks in the fish. If the fish has swallowed the hook deeply into its throat, do not attempt to remove it. These fish have a better chance of survival if the line is cut and the hook is left in.

REVIVING YELLOWFISH:

Help revive the fish. If a released fish do not swim away, hold it in a normal swimming position and gently move it back and forth in the water to move water over the gills and allow oxygen to enter its blood. Most fish recover in a minute or so depending on the temperature of the water and state of the fish. Support the fish from beneath and only release the yellowfish when it has recovered enough to swim away strongly. Resist the urge to get into your next fish before reviving a fish sufficiently. Take your time to have a real look at your yellowfish. Release your fish into a good area, if the current is too swift, use a nearby flat or slow moving, shallow pool as a release area.

WHAT NOT TO USE:	WHAT TO START USING:
Gafs, grips (such as a Bogar grip), hook scales, stomach pumps etc.	Handling towels, de-hookers (such as the ARC-de hooker) knot less landing and recovery nets, measuring tapes rather than scales and finally common sense!

Catch and release fly fishing can be a wonderful way practice the art of flyfishing. And knowing that every release of a fish contributes to the conservation efforts for the Vaal River ensures the future of this species.

SOURCE OF MORE TIPS:

http://www.letsflyfish.com/candr2.htm, http://www.exchile.com/flyfishchile.html, http://www.flyfishingdenver.com/candr.htm http://www.flyfishingspecialist.com/Fly%20Fishing%20Catch%20and%20Release.php, http://www.tpwd.state.tx.us/fishboat/fish/programs/fishrecords/rules/release.phtml http://globalflyfisher.com/fishbetter/10-rules-for-c-and-r/, http://www.dehooker4arc.com/

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Appendix 1: Tips for the sustainable catch, revival and release of Vaal River yellowfish.