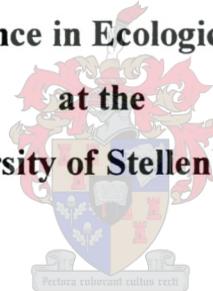


**Assessment of Fish as Bio-Indicators of River Health in Rivers  
of the southwestern Cape**

**Thesis presented in partial fulfilment  
for the degree  
Master of Science in Ecological Assessment  
at the  
University of Stellenbosch**



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**Declaration**

**I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.**

## Abstract

In this study, the Fish Assemblage Integrity Index (FAII) was applied on three rivers within the southwestern Cape. This index uses fish as indicators of biological aquatic integrity and is based on indigenous species expected to be present in biological fish habitats. Fish integrity classes were calculated for each of the sites in the three rivers studied. Sites 2 and 4 within the Lourens River were rated as Class C, whereas sites 1 and 3 were rated as Class F and Class D respectively. Sites 1, 2 and 4 within the Palmiet River were rated Class F, whereas sites 3 and 5 were rated as Class E and Class D respectively. Site 1 within the Hout Bay River was rated as a Class F site, in addition to sites 2 and 3 been rated as Class A. It is however, suggested that the FAII needs to be adjusted to accommodate the general low species richness experienced in the southwestern Cape. In addition to the FAII been applied, the effects of long-term exposure to subtle water quality changes associated with human activities, specifically potential estrogenic compounds in fish from the Lourens River were also investigated. The production of the yolk precursor lipoprotein complex, vitellogenin (Vtg) produced in the liver under estrogen control was employed as biomarker for environmental estrogen exposure. Male fish from the Lourens River were studied using SDS-PAGE gel electrophoresis. Results indicated that 60% of male fish showed the presence of Vtg in their plasma. Abnormal gonad morphology in male and female fish were also assessed using standard histological procedures. Results from this study indicated no observed abnormalities in either male or female gonads. The immediate presence of endocrine disrupters with estrogen activity was investigated by screening water samples from the Lourens, Palmiet and Hout Bay Rivers for estrogen activity. Results indicated that none of the samples appeared to be cytotoxic. In addition, estrogen activity of water samples was also investigated by *in vitro* culturing of water samples with frog, *Xenopus laevis*, liver slices. Results indicated that none of the water samples from the three rivers studied indicated estrogenic activity. Although cytotoxicity and estrogen activity results were negative, the production of Vtg in male fish suggests further research regarding the presence of estrogenic substances in these rivers.

## Uittreksel

In die huidige studie is die 'Fish Assemblage Integrity Index' (FAII) toegepas op drie rivere in die suidwes Kaap. Hierdie indeks gebruik visse as bioindikatore van biologiese akwatiese integriteit en is gebaseer op die inheemse visspesies wat verwag word in biologiese vishabitatte. Integriteitsklasse is bepaal vir elke studieterrrein in die drie rivere wat ondersoek is. 'n Klas C is bepaal vir studieterreine 2 en 4 in die Lourensrivier. Klas F en Klas D is bepaal vir studieterreine 1 en 3 in die rivier onderskeidelik. 'n Klas F is bepaal vir studieterreine 1, 2 en 4 en Klas E en Klas D bepaal vir studieterreine 3 en 5 in die Palmietrivier onderskeidelik. 'n Klas F is bepaal vir studieterrrein 1 in die Houtbaairivier waar 'n Klas A bepaal is vir studieterreine 2 en 3. Dit word egter voorgestel dat die FAII aangepas moet word om die algemene lae spesierykheid wat ervaar word in die suidwes Kaap te akkomodeer. Die reaksie van visse, afkomstig van die Lourensrivier, op die langtermyn blootstelling aan estrogeniese stowwe is ook bestudeer. Spesifieke reaksies van endokriene versteuring, soos vitellogeen (Vtg) produksie in manlike visse is ondersoek deur middel van SDS-PAGE gel elektroforese. Resultate toon dat in 60% van die manlike visse Vtg in die plasma teenwoordig was. 'n Ondersoek na abnormale gonade morfologie in manlike en vroulike visse van die Lourensrivier is deur standard histologiese prosedures gedoen. Resultate hiervan dui op geen sigbare abnormaliteite in die gonades nie. Die onmiddellike teenwoordigheid van endokriene versteurders is bestudeer deur die sitotoksiteit van watermonsters afkomstig van die Lourens, Palmiet en Houtbaai riviere te bepaal. Resultate dui aan dat geen monsters sitotoksies was nie. Die estrogeenaktiwiteit van die watermonsters is ook ondersoek deur van *in vitro* kulture van watermonsters saam met padder, *Xenopus laevis*, lewersnitte gebruik te maak. Geen estrogeniese aktiwiteit is in die watermonsters gevind nie. Al is die sitotoksiteit en estrogeenaktiwiteit resultate negatief, dui die produksie van Vtg in manlike visse op die noodsaaklikheid van verdere navorsing ten opsigte van die teenwoordigheid van estrogeniese stowwe in drie riviere.

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## Chapter 1

### Introduction

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In South Africa, the development of towns and industries and the associated accumulation of waste in built-up areas during the first half of the previous century caused environmental pollution (Department of Water Affairs and Forestry (DWAFF), 1991). South Africa is classified as semi-arid with a below average rainfall (DWAFF, 1993). Furthermore, the country is host to a population of 43,4 people (Barnhoorn, 2001), and experiences an annual population growth of 1.32% (1999 estimated) (Barnhoorn, 2001). In the view of these figures, the demand for fresh water will increase dramatically over the next two decades, as South Africa's population is expected to double by the year 2050 (Davies and Day, 1993). The National Water Act of 1998 requires that the environment is a resource base that needs to be protected; as one of the most limiting natural resources in South Africa is water. This emphasizes the urgent need for careful management and conservation of the scarce water resources in order to maintain their ecological sustainability and fitness for use (Heath and Claassen, 1999).

The River Health Programme (RHP) was developed by DWAFF for exactly this reason, with the main goal of expanding the basis of ecological information on aquatic resources, in order to support the rational management of these systems (Roux, 1997). The present project is intended to contribute to the RHP by providing data that may be used for future management and conservation plans. The RHP makes use of biological response monitoring in order to characterise the response of the aquatic environment to various disturbances. The rationale behind the RHP is that the integrity of the biota inhabiting the river provides a direct, holistic and integrated measure of the integrity of the river as a whole (Karr and Chu, 1997). Biotic integrity is defined as: the ability to support and maintain "a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organisation comparable to that of the natural habitat of the region" (Karr and Dudley, 1981). Communities of fish, aquatic invertebrates and riparian vegetation are the primary indicators used in the RHP (Roux *et al.*, 1999). In order to set a national standard and set procedures to perform assessments of river health, three indices have been developed in South Africa: SASS (South African Scoring Index), a community index for aquatic invertebrates,

RVI (Riparian Vegetation Index) for vegetation and the FAII (Fish Assemblage Integrity Index) for fish.

### *The Fish Assemblage Integrity Index*

The Fish Assemblage Integrity Index (FAII) was developed in 1999 and tested on a number of rivers, including the Crocodile and Elands Rivers, in the Mpumalanga Province. It yielded excellent results for these two rivers (Roux *et al.*, 1999). The index is based on the indigenous fish species expected to be present in biological (fish habitat) segments, which are sections of a river with relatively homogenous fish habitats (Kleynhans, 1999). However, all of the rivers on which the FAII has been applied, occur within the Summer Rainfall Region of South Africa where a higher species richness and lower seasonal variation is generally experienced (Deacon and Kemper, 1999).

The FAII takes into account four aspects of fish assemblages (Kleynhans, 1999):

1. The relative tolerance of the indigenous fish species expected to occur in every segment. Intolerance refers to the degree to which a species is able to withstand changes in environmental conditions. Four components are taken into account, (1) habitat preference and specialisation, (2) food preferences and specialisation, (3) requirement for flowing water during different life stages and, (4) association with habitats with unmodified water quality.
2. Abundance is not included as a measure due to the difficulty in obtaining quantitative information about this aspect. However, it is said that fish species that are the most frequently present in a particular habitat type, also tend to be the most common in habitats where they are found. Therefore, the frequency of occurrence of a species was considered to be a useful metric (Kleynhans, 1999).
3. The rating of general health and well-being. The percentage of fish with externally evident disease or other anomalies is used in the scoring system.
4. Results of the FAII are expressed as a ratio of observed conditions versus conditions that would have been expected in the absence of human impacts.

The sparse knowledge of the responses of certain species to particular aquatic toxins, and the low number of freshwater species in many Western Cape Rivers, limits the use of fish as indicators of ecosystem integrity (Uys *et al.*, 1996). In addition, a natural fish species richness of less than five species is probably not amendable to assessment with the FAII (Kleynhans, 1999). The assessment of fish populations in the Fynbos Biome is, however, of the utmost importance as, 16 of the 19

species (84%) found in this region are endemic (Impson *et al.*, 1999). The region is known to have the greatest concentration of endemic freshwater fish in southern African (Skelton, 1993), and the majority (73%) of these species are threatened. Alarmingly, 12 species are endangered or critically endangered (Impson *et al.*, 1999), due to predation by and competition with invasive alien fish and habitat degradation and destruction by inappropriate agricultural development (Davies and Day, 1986). These figures stress the urgent need for the FAII to be adjusted such that it would become more applicable to conditions (e.g. low species richness) experienced in the Fynbos Biome.

### *The use of fish as bio-indicators*

Fish taxa are selectively tolerant or sensitive to altered flow regimes, deterioration in water quality, and habitat alteration (Barnhoorn, 2001). Certain stresses can result in a decrease in species richness, or a dominance of a certain species, while other stresses may drastically reduce biomass or productivity (Hocutt, 1984; Uys *et al.*, 1996). When an aquatic system is polluted, fish can thus be affected directly or indirectly. The direct effects are initiated at the lower level of biological organisation (molecular or sub-cellular level). These effects of the pollutant propagate upwards through the levels of biological organisation and affect physiological and metabolic processes (Barnhoorn, 2001). Ultimately these can be manifested as changes at the population and community levels (Thomas, 1990), and may in the long term thus influence the FAII score for a specific river or a segment within a river.

When fish are affected indirectly, the effects can be seen as changes in the food chain of fish, or the behaviour of the fish. When fish are subjected to stress (e.g. water pollution) they may experience instant neuro-endocrinal changes (primary response) that produce secondary biochemical and physiological changes (e.g. hormone disruption). These changes ultimately lead to responses at individual, community and population levels in terms of growth, survival and reproduction (Barnhoorn, 2001).

Because aquatic contamination may affect fish to such a large extent, fish may act as excellent biological indicators of aquatic pollution or the exposure to such contamination. Bio-indicators are used to assess the health status of organisms and to provide early-warning responses of environmental risks (Payne *et al.*, 1987). Fish as bio-indicators for biological monitoring have a high public profile and consequently are used extensively for conservation status and bioaccumulation studies (Heath and Claassen, 1999). In aquatic ecosystems, fish or fish

communities are regarded as indicators of overall system health (Barnhoorn, 2001). Fish are therefore good organisms to use in biomonitoring because (Barnhoorn, 2001):

- They are relatively long-lived and mobile, and are thus good indicators of long-term effects and habitat changes.
- Fish communities usually indicate a range of species representing a variety of trophic levels.
- Fish are placed high up in the aquatic food chain and are eaten by man, making them important subjects for assessing contamination.
- Fish are relatively easy to collect and identify to species level.
- By using fish as bio-indicators, one may avoid unacceptable and often irreversible effects such as mortality.

Low species richness and a poor knowledge of individual species responses to specific contaminants may, however, limit the use of fish as bio-indicators of ecosystem integrity (Uys *et al.*, 1996).

#### *Endocrine disruption in fish*

Numerous studies indicated that contaminants may cause dramatic biomarker responses, for example, the induction of detoxification enzymes, organ dysfunction, reduced fish community integrity and impaired reproduction (Adams *et al.*, 1999). All of these are non-specific responses. Different contaminants may induce different responses in bio-indicators. Endocrine disrupters mimic human hormones, interfering with the basic functions of the body such as metabolism, growth, immune response and reproductive functions (Kime, 1998; Van Der Kraak *et al.*, 2001; Knorr and Braunbeck, 2002). An endocrine disrupter is an exogenous substance or mixture that alters functions of the endocrine system and consequently could potentially cause adverse health effects in an intact organism, or its progeny or populations (Sole *et al.*, 2001). Estrogen and/or estrogen mimics are released into aquatic systems in numerous ways: agricultural runoff, as organic waste and through wastewater treatment facilities (Eaton and Lydy, 2000).

Much of the discussion around endocrine disruption has, to date, revolved around manmade chemicals. Earlier studies linked polychlorinated biphenyls and some pesticides to reproductive problems in fish-eating birds and changes in fish reproductive organs (Christen, 1998; Knorr and Braunbeck, 2002). A decrease in the rate and intensity of male guppy sexual display after exposure to natural estrogen (17 $\beta$ -estradiol) and the xenoestrogen (4-*tert*-octylphenol), has been observed

(Bayley *et al.*, 1999). This was supplemented by a decline in reproductive success and ultimately resulted in sex reversal (Knorr and Braunbek, 2002). Even though there appear to be more examples of endocrine disruption in fish than in other wildlife groups, there is little evidence to support the idea that fish are more sensitive to the effects of estrogen disrupters than other wildlife (Van der Kraak *et al.*, 2001).

Vitellogenin (Vtg), a liver-derived estrogen-induced lipoprotein, is normally produced only by oviparous female fish as a response to estrogens circulating in the plasma (Sole *et al.*, 2001), and transported to the growing oocytes to form the major constitute of yolk (Holbech *et al.*, 2001). Although estrogens are steroid hormones associated with the female reproductive system, male non-mammalian species exhibit estrogen receptors in the liver and the capability to produce Vtg. This suggests that estrogenic compounds enter the environment at concentrations that could cause ecological major effects (Christen, 1998), since male fish are capable of producing Vtg in response to exogenous estrogens and xenoestrogens (Holbech *et al.*, 2001). Thus, since Vtg may be produced by males, Vtg production has the potential for employment as a specific biomarker for environmental estrogenic activity (Monteverdi and Di Giulio, 1999; Sheahan *et al.*, 2002). Among the palliological effects of increased vitellogenesis in male fish are kidney damage, increased susceptibility to illnesses, decreased testicular growth, decreased spermatogenesis and changes in sexual maturity (Sole *et al.*, 2001). All of these clearly have negative consequences on reproduction and are therefore ecologically highly relevant. Estrogenic compounds have also been found to cause a decrease in testosterone levels in males (Gunderson *et al.*, 2000), changes in gross male gonads (Bayley *et al.*, 1999) and a substantial increase in the liver somatic index (LSI) (Donohoe *et al.*, 1999). This, together with the fact that male fish can potentially produce vitellogenin suggests that feminisation may take place after exposure to estrogenic substances. This may cause major disruptive changes to fish assemblages, and has been confirmed by a high prevalence of the female phenotype observed in various rivers (Metcalf *et al.*, 2001) and high incidences of intersexuality and hermaphroditism (Sole *et al.*, 2001).

The southwestern Cape has a mediterranean climate characterised by hot, dry summers and wet, colder winters. The area boasts extensive agricultural activity including fruit orchards and the growing of grapes for wine and export. Agricultural runoff of estrogen mimics may cause endocrinal disruption in fish from southwestern Cape rivers, thus lowering river health.

### *Aims and Objectives*

The main aim of this study is to assess piscine river health of the Palmiet, Hout Bay and Lourens Rivers. More specifically, the objectives include:

- To apply the FAII to the three rivers in order to determine the integrity of these rivers.
- To critically evaluate the general use of the FAII within the southwestern Cape where a low diversity in fish species are found.
- To determine whether vitellogenin production occurs in male fish in the Lourens River, which could indicate possible exposure to estrogenic substances.
- To assess other effects of estrogenic exposure in male fish in the Lourens River, for example changes in gross gonad morphology.
- To investigate possible estrogen activity and the cytotoxicity of water samples from the Lourens, Palmiet and Hout Bay Rivers.
- To compare and test the FAII results against estrogenicity results.

It is expected that segments of rivers downstream from agricultural activity and industrial or urban areas will show a lower relative FAII score (%), and will thus be less healthy than segments upstream from these developments. Since the FAII was developed for Summer Rainfall Areas with relatively high species richness, it is expected that some recommendations will have to be made in order to make the FAII more applicable to rivers of the southwestern Cape. These rivers have highly fluctuating water levels and flow regimes, mainly due to the dominant winter rainfall experienced in the region. It is also expected that male fish in river segments downstream from any development might show effects of exposure to estrogenic substances. They might well produce vitellogenin, and thus display feminisation and abnormal gonad morphology.

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## Chapter 2

### Assessing fish population integrity in three rivers of the southwestern Cape using the Fish Assemblage Integrity Index

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#### *Introduction*

Due to the current human population growth of 1.32% (Barnhoorn, 2001) and an increase in environmental pollution, the demand for fresh water will increase dramatically over the next two decades (Davies and Day, 1993). Since South Africa is classified as semi-arid with a below average rainfall (Department of Water Affairs and Forestry (DWAF), 1993), the urgent need for careful management and conservation of the scarce water resource is emphasised. This is essential in order to maintain ecological sustainability of water and its fitness for use (Heath and Claassen, 1999). The River Health Programme (RHP) was developed by DWAF for exactly this reason, with the main goal to expand the ecological basis of information on aquatic resources in order to support the rational management of these systems (Roux, 1997). River health is defined as the degree to which three main attributes of a river (its energy source, water quality and flow regime) plus its biota and their habitats, match the natural condition at all scales (Karr, 1991). This study, which forms part of the RHP, will thus assist in collating information on the health of the Palmiet, Hout Bay and Lourens Rivers, to assist in the implementation of better management and conservation strategies of these rivers. To date, no fish-related studies have been conducted on the Hout Bay River. This study will thus provide baseline data for future studies, specifically regarding the Hout Bay River.

The RHP makes use of instream biological response monitoring in order to characterise the response of the aquatic environment to various disturbances. The rationale behind the RHP is that the integrity of the biota inhabiting the river provides a direct, holistic and integrated measure of the integrity of the river as a whole (Karr and Chu, 1997). Biotic integrity is defined as the ability to support and maintain “a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organisation comparable to that of the natural habitat of the region” (Karr and Dudley, 1981). Communities of fish, aquatic invertebrates and riparian vegetation are the primary indicators used in the RHP (Roux *et al.*, 1999). In order to set a national standard and set procedures to perform biological assessments of river health, three indices have been

developed (Kleynhans, 1999): SASS (South African Scoring Index), that is a community index for aquatic invertebrates, RVI (Riparian Vegetation Index) for vegetation and the FAII (Fish Assemblage Integrity Index) for fish.

The Fish Assemblage Integrity Index (FAII), developed in 1999 (Roux *et al.*, 1999), is based on the indigenous fish species expected to be present in biological (fish habitat) segments. These segments are sections of a river with relatively homogenous fish habitats (Kleynhans, 1999). The FAII has the potential to provide qualitative, descriptive criteria for the desired ecological integrity of rivers for management purposes, in terms of the new South African Water Law (Kleynhans, 1999). The FAII takes into account three aspects of fish assemblages (Kleynhans, 1999):

1. The relative tolerance of the indigenous fish species expected to occur in every segment. Tolerance refers to the degree to which a species is able to withstand changes in the environmental conditions under which it occurs.
2. Abundance is not included as a measure due to the difficulty in obtaining quantitative information on this aspect. Frequency of occurrence of a species was considered to be a useful metric, since fish species most frequently present in a certain habitat, also tend to be the most common in habitats where they are found (Kleynhans, 1999).
3. The percentage of fish with externally evident disease or other abnormalities is used in the scoring system to rate the general health and well-being.
4. Results of the FAII are expressed as a ratio of observed conditions versus condition that would have been expected in the absence of human impacts.

All of the rivers on which the FAII has to date been applied are located in Summer Rainfall Areas (e.g. Crocodile and Elands Rivers) where, a higher species richness and lower seasonal variation is generally experienced (Deacon and Kemper, 1999). The sparse knowledge of the responses of certain species to particular toxins, and the low number of freshwater species in most areas, place limits on the use of fish as indicators of ecosystem integrity (Uys *et al.*, 1996). Additionally, a natural low fish species richness is most likely not compliant to assessment with the FAII (Kleynhans, 1999). The southwestern Cape has a low diversity of indigenous freshwater fishes but is known for the greatest concentration of endemic freshwater fish in southern African (Skelton, 1993). It is thus important to test and perhaps adjust the FAII such that it can be effectively applied here.

Eighty four percent of the fish species within the Cape Floristic Region are endemic, of which the majority are threatened. Disturbingly, 12 species are endangered or critically endangered (Impson *et al.*, 1999). Negative impacts likely to be responsible for threats to the indigenous fish populations include invasive alien species, habitat degradation and habitat destruction by inappropriate agricultural development (Davies and Day, 1986). The figures quoted above also stress the importance of adjusting the FAII to become more applicable to conditions (e.g. low species richness and higher seasonal variation) experienced in the Cape Floristic Region.

The aim of this study is to assess river health of the Palmiet, Hout Bay and Lourens Rivers by applying the FAII. More specifically, the objectives include aspects such as: 1) To apply the FAII to the three rivers, and thus to determine the integrity of these rivers and, 2) to critically evaluate the general use of the Fish Assemblage Integrity Index in rivers of the southwestern Cape which are known to contain a relatively low species richness.

## ***Materials and Methods***

### *Study area*

The study area comprises the Hout Bay, Palmiet and Lourens Rivers (Figure 1). The three rivers occur within the Western Cape Province and, because they occur in the Winter Rainfall Region, have a lower species richness (Tharme *et al.*, 1997) and a higher seasonal variation is experienced (Table 1) than in the rivers in Summer Rainfall Regions. Three segments in the Hout Bay River, four in the Lourens Rivers and five in the Palmiet River, were chosen as study sites (Table 2). These segments were chosen such that they would provide an overall representation of all the different rivers, and are characterized by different habitats and flow regimes. The study area was sampled during autumn (April and May 2002) and spring (September 2002) to incorporate the possible effects of seasonal flux.

**Table 1.** Abiotic characteristics of the Palmiet, Hout Bay and Lourens Rivers to be studied (Tharme *et al.*, 1997; Brown and Day, 1998).

(\* = data not available at present).

River	Catchment (km <sup>2</sup> )	Area Length (km)	Mean annual rainfall (mm)	Mean annual runoff (m <sup>3</sup> )
Palmiet	465	74	1139	310 x 10 <sup>6</sup>
Hout Bay	33.8	12	1457	*
Lourens	92	20	915	21 x 10 <sup>6</sup>

Although all three rivers experience agricultural runoff (including potential pollutants such as pesticides, herbicides and fertilizers) and dumping of sewage and industrial waste products, Site 1 in all three rivers falls outside the impact range of dams and irrigation. The rivers, and more specifically all the different sites within the rivers (except site 1), are thus expected to experience various degrees of disturbance.

**Table 2.** Location of the different sites per river sampled

River	Site no	Location	Descriptive locality
Lourens	Site 1	34° 01.741' S; 18° 57.407' E	Lourensford Estate (IFR site 1)
	Site 2	34° 04.502' S; 18° 53.341' E	Vergelegen Estate (IFR site 2)
	Site 3	34° 04.983' S; 18° 32.144' E	Radloff Park (IFR site 3)
	Site 4	34° 05.775' S; 18° 49.772' E	Victoria Road, Strand (IFR site 5)
Palmiet	Site 1	34° 03.351' S; 19° 02.465' E	Nuweberg Forest (IFR site 1)
	Site 2	34° 07.102' S; 19° 01.481' E	Grabouw town centre
	Site 3	34° 14.641' S; 18° 59.666' E	Arieskraal Farm (IFR site 2)
	Site 4	34° 17.185' S; 18° 58.701' E	Kogelberg Nature Reserve (IFR site 3)
	Site 5	34° 19.241' S; 18° 58.170' E	Kogelberg Nature Reserve (IFR site 4)
Hout Bay	Site 1	33° 58.449' S; 18° 24.601' E	Upstream from Hely Hutchinson Dam
	Site 2	34° 00.931' S; 18° 22.863' E	Disa Road Bridge, Hout Bay
	Site 3	34° 01.758' S; 18° 21.231' E	Victoria Road Bridge, Hout Bay

### *Fish collection*

The Lourens and Palmiet Rivers were both sampled during late autumn and early spring, to facilitate documentation of seasonal variation. The Hout Bay River was, however, only sampled in spring. Fish were sampled using electro-fishing and a seine net (3.0 m x 1.5 m). The fish species and abundance per species caught in the different flow-depth habitats (Table 3), as well as the sampling method and effort, were noted and entered into the Rivers 2000 database. In addition, flow conditions, water quality, fish velocity-depth classes present, cover present, instream and surrounding land use and habitat integrity of the specific sites, were noted and entered into the same database.

**Table 3.** Habitats sampled and cover types available at sites in the Lourens, Palmiet and Hout Bay Rivers. OV = Overhanging vegetation, UB = Undercut banks and root wads, SS = Substrate, AM = Aquatic macrophytes and NS = Habitats not sampled. A '+' indicates the presence of the variables, and '-' an absence thereof.

River	Flow-depth habitats															
	Slow-deep				Slow-shallow				Fast-deep				Fast-shallow			
	OV	UB	SS	AM	OV	UB	SS	AM	OV	UB	SS	AM	OV	UB	SS	AM
<b>Lourens</b>																
1	+	+	+	-	+	+	+	-	+	-	+	-	+	+	+	+
2	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
<b>Palmiet</b>																
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	NS	NS	NS	NS	+	-	+	-	NS	NS	NS	NS	NS	NS	NS	NS
3	+	+	+	+	+	+	+	+	NS	NS	NS	NS	+	+	+	+
4	+	+	+	+	+	+	+	+	NS	NS	NS	NS	NS	NS	NS	NS
5	+	+	+	+	+	+	+	+	NS	NS	NS	NS	+	+	+	+
<b>Hout Bay</b>																
1	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
2	+	+	+	+	+	-	+	+	-	-	-	-	-	+	+	+
3	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-

### *Application of the FAII*

The scoring system developed by Kleynhans (1999), incorporated in the Rivers 2000 database, was used to determine the overall relative FAII scores for the three rivers. Intolerance, preference, abundance and health of the different species expected and observed were entered and taken into account in further analyses. Data on the intolerance and preference of expected and observed fish species in each segment were obtained from the Dr Neels Kleynhans. These data are based on subjective expert knowledge, obtained over a long period under different conditions, and were thus not obtained scientifically (N. Kleynhans, *pers. comm.*).

Ratings with regard to intolerance included trophic and habitat specialization, flow requirement, requirement of unmodified water quality and an overall intolerance rating. Ratings were scored as follows:

- 1-2 = Tolerant
- 2-3 = Moderately tolerant
- 3-4 = Moderately intolerant
- 4-5 = Intolerant

Preference ratings with regard to four flow-depth habitats were also entered. These habitat categories include slow-deep (<0.3 m/s; >0.5 m), slow-shallow (<0.3 m/s; <0.5 m), fast-deep (>0.3 m/s; <0.3 m) and a fast-shallow (>0.3 m/s; <0.3 m) variants.

For each flow-depth habitat, the presence and preference of overhanging vegetation, bank undercuts and substrate, macrophytes and water column were scored. Ratings were scored as follows:

- 0-1 = No preference, irrelevant
- 1-2 = Low preference
- 2-3 = Moderate preference
- 3-4 = High preference
- 4-5 = Very high preference

For each species expected to be present in a depth-flow habitat of a site, the expected frequency of occurrence was estimated and the observed frequency of occurrence calculated. The frequency of the fish species expected and observed was rated as follows:

- infrequent occurrence = 1
- frequent occurrence = 3
- widespread occurrence = 5

The health of the fish species expected and observed at each site was calculated as follows:

- frequency of affected fish >5%, score = 1
- frequency of affected fish 2 - 5%, score = 3
- frequency of affected fish <2%, score = 5

Cape galaxias (*Galaxias zebratus*) was expected in the slow-shallow and slow-deep habitats, at Sites 1 and 2 of the Lourens, Palmiet and Hout Bay Rivers (D. Impson, *pers. comm.*; Skelton, 1993). *G. zebratus* and the Cape Kurper (*Sandelia capensis*) were expected in the same habitats of the remaining sites (D. Impson, *pers. comm.*; Skelton, 1993).

The expected and observed FAII values for the different flow-depth habitats of the different sites were calculated by the Rivers 2000 database by applying the following equation:

$$\text{FAII value (expected and observed)} = \Sigma IT \times ((F + H)/2)$$

**IT** = intolerance rating for individual species expected to be present in a fish habitat segment in habitats that were sampled.

**F** = expected frequency of occurrence rating for individual species expected to be present in a fish habitat segment at sites that were sampled.

**H** = expected health rating for species expected to be present.

The relative FAII score for each flow-depth habitat was calculated as follows:

$$\text{FAII score} = \text{FAII value (observed)} / \text{FAII value (expected)}$$

The average of the FAII scores obtained for the different flow-depth habitats were used to interpret the obtained values and to class the different sites (Table 4).

**Table 4.** FAII assessment classes (Kleynhans, 1996).

Class rating	Description of generally expected conditions for integrity classes	Relative FAII score (% of expected)
A	Unmodified, or approximate natural conditions closely	90 to 100
B	Largely natural with few modifications. A change in community characteristics may have taken place, but species richness and presence of intolerant species indicate little modification	80 to 89
C	Moderately modified. A lower than expected species richness and presence of most intolerant species. Some impairment of health may be evident at the limit of this class.	60 to 79
D	Largely modified. A clearly lower than expected species richness and absence or much lowered presence of intolerant species. Impairment of health may become more evident at the lower limit of this class.	40 to 59
E	Seriously modified. A strikingly lower than expected species richness and general absence of intolerant species. Impairment of health may become very evident.	20 to 39
F	Critically modified. An extremely lowered species richness and an absence of intolerant and moderately intolerant species. Only tolerant species may be present with a complete loss of species at the lower limit of the class. Impairment of health generally very evident.	0 to 19

## Results

### *Fish health ratings*

Less than 2% of the fish caught indicated any evident anomalies. Consequently, all fish were considered to be in good health. Anomalies observed included a parasitic infection, black spot and a fungal infection known as fin rot.

### *Lourens River*

The indigenous species expected in the Lourens River were *G. zebratus* at Sites 1 and 2 and, *G. zebratus* and *S. capensis* at the remaining sites. During surveys *G. zebratus* was observed at sites 2—4 and *S. capensis* at only site 4 (Table 5). Exotic species observed were *Tilapia sparrmanii* at Sites 3 and 4 and rainbow trout (*Onchorhynchus mykiss*) at Site 1 of the Lourens River (Table 5).

**Table 5.** The total number of individuals of every fish species observed at sites 1 to 4 of the Lourens River, during the autumn and spring surveys. Asterisks indicate exotic fish species observed.

Species	Intolerance rating	Sites			
		1	2	3	4
<i>Galaxias zebratus</i>	2.6	-	14	34	4
<i>Sandelia capensis</i>	2.3	-	-	-	22
<i>Tilapia sparrmanii</i> *	1.3	-	-	23	2
<i>Onchorhynchus mykiss</i> *	3.4	12	-	-	-
Relative FAII score (%)		0	67	43	62
Integrity class rating		F	C	D	C

### Site 1

The indigenous species *G. zebratus* was unexpectedly found to be absent from this site during the autumn and spring surveys and the relative FAII score was rated as Class F (critically modified, or an extremely lowered species richness and an absence of intolerant and moderately intolerant indigenous species), despite the apparent excellent general condition of the habitat. Nevertheless, habitat sensitive rainbow trout *O. mykiss*, were observed at this site during spring and autumn.

### Site 2

At this site *G. zebratus* was observed in both autumn and spring in the slow-shallow water habitat. During the autumn survey this species was absent from the slow-deep water habitat and in spring this habitat no longer existed at this site due to lowered water levels. However, the relative FAII score was rated as Class C (Moderately modified). During the spring survey, numerous juvenile ( $\pm 1$  cm) *G. zebratus* were observed in standing pools, which might have had an elevating effect on the relative FAII score.

### Site 3

The expected species *S. capensis* was found to be absent from this site. However, *G. zebratus* was observed in the slow-deep habitat in autumn and in both slow-deep and slow-shallow water habitats in spring. The relative FAII score was rated as Class D (Largely modified). Although absent in spring, high numbers of the exotic species *T. sparrmanii* were observed in autumn.

### Site 4

Both *G. zebratus* and *S. capensis* were observed at this site in the slow-shallow and slow-deep water habitats during the spring survey, whereas only *S. capensis* was observed in autumn. *T. sparrmanii* was also observed during autumn and spring, and might be the reason for the low numbers of *G. zebratus* observed as *T. sparrmanii* is also predatory. The relative FAII score was rated as Class C (Moderately modified).

### *Palmiet River*

The indigenous species expected in the Lourens River varied from *G. zebratus* at sites 1—2 and, *G. zebratus* and *S. capensis* at the remaining sites. *G. zebratus* was found to be absent from the Palmiet River at the sites assessed (Table 6). However, *S. capensis* was observed at Sites 3 and 5 (Table 6). The exotic species *Micropterus dolomieu* (small-mouthed bass) was observed at Site 4 (Table 6).

**Table 6.** The total number of individuals observed at Sites 1 to 5 of the Palmiet River during the autumn and spring surveys. Asterisks indicate exotic fish species observed.

Species	Intolerance rating	Sites				
		1	2	3	4	5
<i>Sandelia capensis</i>	2.3	-	-	3	-	4
<i>Micropterus dolomieu</i> *	2.3	-	-	-	1	-
Relative FAII score (%)		0	0	38	0	49
Integrity class rating		F	F	E	F	D

### Site 1

Although this is the only site where the two endemics *G. zebratus* and *S. capensis* may still be found (Brown and Day, 1998), no fish were observed at this site and the relative FAII score was thus rated as Class F (Critically modified). The occurrence of a weir immediately downstream from this site might prevent upstream fish migration, however, no fish were observed below the weir either.

### Site 2

No fish were observed at this site, and the relative FAII score was therefore rated as Class F (Critically modified). Difficulties experienced in sampling this site included a high water level (> 1.5 m), high turbidity and a strong water flow, which resulted in only the slow-shallow flow-depth habitat being sampled. The possibility thus exists that fish species are in fact present at this site, in flow-depth habitats not sampled.

### Site 3

Although *G. zebratus* was found to be absent, *S. capensis* was observed at this site in both autumn and spring. The Arieskraal Dam approximately 2 km upstream and a weir-like structure immediately downstream from the site, might be responsible for the lower than expected species richness observed. The relative FAII score for this site was rated as Class E (Seriously modified), but close to Class D (37.7%).

#### Site 4

The expected indigenous species *G. zebratus* and *S. capensis* were found to be absent from this site, consequently the FAII score was rated as Class F (critically modified). During the autumn survey, a juvenile individual of the exotic species *M. dolomieu* was caught. The possibility thus exists that this and other exotic species previously observed occur in the deep, inaccessible water and might be responsible for the absence of indigenous species.

#### Site 5

Although *G. zebratus* was found to be absent, *S. capensis* was observed at this site in both autumn and spring, in both slow-shallow and slow-deep water habitats. The relative FAII score for this site was rated as Class D (Largely modified).

#### *Hout Bay River*

The indigenous species expected at the Lourens River varied from *G. zebratus* at sites 1—2 and, *G. zebratus* and *S. capensis* at the remaining sites (Table 7). *G. zebratus* and *S. capensis* were observed at Sites 2 and 3 (Table 7). Also, an unexpected indigenous species *Gilchristella aestuaria* (Estuarine herring), an estuarine fish, was observed at Sites 2 and 3 (Table 7). No exotic fish were observed in the Hout Bay River.

**Table 7.** The total number of individuals of every fish species expected to be present at sites 1 to 3 Hout Bay River, and as was observed during the autumn and spring surveys.

Species	Intolerance rating	Sites		
		1	2	3
<i>Galaxias zebratus</i>	2.6	-	4	5
<i>Sandelia capensis</i>	2.3	-	14	5
<i>Gilchristella aestuaria</i>	2.7	-	1	10
Relative FAII score (%)		0	100	98
Integrity class rating		F	A	A

#### Site 1

Although this site provided sufficient habitat, no fish were observed at this site and the relative FAII score was rated as Class F (Critically modified). The series of dams immediately downstream from this site might be responsible for the lack of fish species observed, as fish might not be able to migrate upstream.

### Site 2

Both *G. zebratus* and *S. capensis* were observed at this site in the slow-shallow and slow-deep water habitats. Consequently, the FAII score was rated as Class A (Unmodified). *S. capensis* was found to have a widespread distribution, whereas *G. zebratus* was found to occur more infrequently. One individual of the indigenous species *G. aestuaria* was also caught at this site. Since both the expected fish species were observed in the expected habitats, the presence of *G. aestuaria* did not influence the rating of the FAII score.

### Site 3

Both *G. zebratus* and *S. capensis* were observed at this site in the slow-shallow and slow-deep water habitats during spring. These species were less common than at Site 2. High numbers of the species *G. aestuaria* were also observed at this site. The presence of this unexpected, indigenous species influenced the FAII score positively, and it was rated as Class A (Unmodified). With the exclusion of *G. aestuaria*, the FAII score was rated as Class C (Moderately modified).

## ***Discussion***

### *Relative FAII score per segment*

In the Lourens River, a general increase in the relative FAII score was observed in a downstream direction (Table 5). It was expected that the relative FAII score would decrease with an increase in potential impacts on river health. In fact, exactly the opposite was observed for the Lourens River. The Palmiet River is known to have a depauperate fish fauna (Brown and Day, 1998) and, fitting this trend, no fish were observed at sites 1 and 2 of this river; consequently a FAII score of zero is observed. One individual exotic fish species was caught at site 4, which caused the score at this site to be zero. The relative FAII score for site 5 was found to be the highest of all the sites. Although an increase in the relative FAII score was not observed for the Palmiet River (as observed at the Lourens River) again the opposite of what was expected was observed. At site 1 of the Hout Bay River no fish were observed, and a score of zero was calculated. Site 2 of this river was found to have the highest score, since both of the expected species were observed in high numbers in their expected habitats. At site 3, *G. zebratus* was observed in all of the expected habitats, whereas *S. capensis* was observed in only one of the expected habitats. Once again, the opposite of what was expected was found at the Hout Bay River.

### *River integrity*

#### Lourens River

Earlier studies indicated that two introduced species dominating the fish community of the Lourens River (Tharme *et al.*, 1997). The headwaters were dominated by *O. mykiss*, whereas *T. sparrmanii* was found to be the most common species in lower areas. In this study, the same results were obtained with regard to *O. mykiss*, whereas *T. sparrmanii*, although observed at two sites, was not found to be a dominant species. Although suitable habitats for fish occur at most sites examined in the Lourens River, results from this study indicated that only one of the expected indigenous species, *G. zebratus*, was commonly present. Significantly, this species was not found in the mainstream of the Lourens River in the earlier study by Tharme *et al.* (1997). In the current study, high numbers of *S. capensis* was only observed at site 4, where it appears to be the dominant species. Both *G. zebratus* and *S. capensis* are considered to have a high conservation value, in that the Lourens River populations might be genetically distinct from other populations, due to a long period of isolation (Tharme *et al.*, 1997). In addition, both the expected indigenous species were observed at site 4. This indicates that this segment of the river may be of high conservation value.

The lower abundance of the moderately tolerant *S. capensis* may be attributed to poorer habitat quality at downstream locations. Introduced *T. sparrmanii* may also raid *S. capensis* nests during spawning time to prey on eggs and embryos, thus affecting the abundance of this indigenous species (Brown and Day, 1998). In addition, the lower number of *S. capensis* might be responsible for the greater abundance of *G. zebratus*, since this species may predate on the smaller *G. zebratus* (Brown and Day, 1998). This possibility was confirmed by the abundance of juvenile ( $\pm 1$  cm) *G. zebratus* that were observed at two sites during the spring survey. The presence of the predatory exotic species *O. mykiss* (Site 1) might be responsible for the absence of *G. zebratus* in the headwaters. It is unlikely that the larger *S. capensis* would have occurred naturally in this area, since it prefers slow flowing water with plant and root cover (Skelton, 1993). Furthermore, site 1 was rated as Class F, due to the absence of *G. zebratus* and the presence of *O. mykiss*. Nevertheless, rainbow trout are considered to be an intolerant species, with a mean intolerance rating of 3.4, due to its water quality requirements and preference for cool, clear water (Plafkin *et al.*, 1989). Its presence is thus indicative of good water quality and suitable cover in fast-flowing water.

In total, the indigenous fish community of the Lourens River seems to have improved since the previous study in 1997. With the exception of site 1, indigenous species seem to dominate and the exotic species *T. sparrmanii*, which had previously dominated downstream areas (Tharme *et al.*, 1997), was found in much lower numbers and at less sites. In addition, another exotic species *Cyprinus carpio* (mirror carp), previously observed in the Lourens River (Tharme *et al.*, 1997), was not observed during this survey. The increase in the abundance of indigenous species can partly be attributed to the reasonable condition of the river, with regard to river morphology. Although water quality may be poor in the downstream urban segments of the river, it is still adequate for the survival and reproduction of these species. However, the presence of *O. mykiss* in the headwaters might make it impossible for *G. zebratus* to thrive. Furthermore, the obstruction by downstream dams and weirs might make it impossible for this species to re-colonize these areas.

### Palmiet River

Floods, high water levels and a general high baseflow complicated the current survey of the Palmiet River. This led to certain habitat types not being suitable for sampling. Although this river is known to have a depauperate fish fauna, it does not lessen its importance, especially with regard to species such as *S. capensis* and *G. zebratus* (Brown and Day, 1998). Populations of these species have been isolated from those in other rivers over geological time scales (Brown and Day, 1998) resulting in a potential unique genetic make-up for these two species. Few fish were observed in

this river during both autumn and spring. Two sites yielded no fish whereas three sites only delivered minimal numbers of a single species. *G. zebratus* was found to be absent from the Palmiet River, whereas *S. capensis* was observed at only two sites. At a single site, one juvenile individual of the invasive alien species *M. dolomieu* was observed.

Once an alien fish species establishes itself, it becomes almost impossible to eradicate, since biological control agents and poisons cannot be administered throughout a system (Impson *et al.*, 1999). The impact of invasive alien fish is possibly the greatest threat to the existence of indigenous species in the Cape Floristic Region (Impson *et al.*, 1999). This is supported by results obtained from the current survey of the Palmiet River. Although only a single individual from one exotic species was observed, it cannot be excluded that this species is more widely distributed. In a previous study, in addition to *M. dolomieu*, other such exotics included *M. salmoides* and *Lepomis macrochirus* (bluegill sunfish). It is thus likely that more exotic species occur in the Palmiet River than were found during the present study, since all habitats could not be sampled within the limits of the present study.

The introduction of the three exotic species, especially *L. macrochirus* and *M. dolomieu*, are likely to have had more of an impact on the along stream distributions of the endemic *S. capensis* and *G. zebratus* than dams or other anthropogenic influences (Brown and Day, 1998). These three exotics, which thrive in the slower flowing middle and lower reaches of the Palmiet River, are larger and faster growing than the indigenous species. Their piscivorous feeding habits, appear to have excluded *G. zebratus* at all the sites. These exotics may thus also be responsible for the limited number of *S. capensis* observed at only two sites. This is a disturbing possibility, since records from the 1970's indicated that the distribution of these exotic and indigenous species did not overlap. Presently, it appears that the exotic species have exterminated indigenous populations, by excluding their access to refuges due to obstructions such as various dams and weirs in the river.

### Hout Bay River

No fish were observed at site 1 of the Hout Bay River, however, a single individual of *G. zebratus* was observed during a different survey in autumn (D. Ollis, *pers. comm.*). The present survey yielded both the expected indigenous species *G. zebratus* and *S. capensis* at the remaining two sites. An additional indigenous species, *G. aestuaria*, was also observed at these sites. The presence of this species had an elevating effect on the FAII rating (Class A) of site 3. In the absence of this latter species, the rating would have been Class C.

*G. aestuaria* occurs in estuaries and freshwaters, where it breeds throughout the year (Skelton, 1993). This species is a valuable link in the natural foodchain between small crustaceans and larger predatory fish such as *S. capensis*. It is classified as moderately tolerant, due to an intolerance rating of 2.7. The presence of three indigenous species, in addition to the apparent present absence of exotic species, may indicate that no invasive alien species occur in this river. The Hely Hutchinson Dam immediately downstream from site 1, as well as a series of other dams and weirs, most probably prevents indigenous species like *G. zebratus* and *S. capensis* from colonizing the headwaters of the river.

To date, no published studies have been done on the fish fauna of the Hout Bay River. This survey thus provides baseline data for future studies. Such studies are recommended as the Hout Bay River was only sampled in spring, due to time constraints. Seasonal variation was thus not assessed. Nevertheless, it seems as if the integrity of the fish community of the Hout Bay River is still intact. Although water quality may be poor in the downstream urban segments of the river, it still seems adequate for the survival and reproduction of *G. zebratus* and *S. capensis*.

#### *Observed limitations of the FAII*

The preference and intolerance ratings for the different fish species, with regard to assorted variables, are critical in the calculation of the FAII score. Most of these ratings are based purely on expert knowledge and opinions (N. Kleynhans, *pers. comm.*). It is likely that a combination of various factors such as water quality and habitat suitability influence the distribution and presence of fish, rather affecting the distribution and presence independently. These ratings, being purely subjective, might thus not reflect the true impact of various factors on the distribution and presence of fish.

In most cases, the FAII is an under-estimation of the biological integrity of a segment (Kleynhans, 1999). Due to river morphology, certain habitats in the Palmiet River were not suitable for sampling (Table 4) despite the fact that it is highly possible that fish do occur in these habitats. The FAII does not take the ability to sample certain habitats into account. Furthermore, certain species such as the longfin eel (*Anguilla mossambica*), which occurs in rivers of the southwestern Cape, cannot be sampled by methods such as electro-fishing and seine nets. Together these factors may increase the under-estimation of the integrity of a specific segment.

At site 1 on the Lourens River, no indigenous species were observed, possibly due to the presence of the exotic species *O. mykiss*. Consequently, the FAII score was rated as Class F. According to Table 3, this class represents a critically modified segment with an absence of intolerant species and an evident impairment of health. However, *O. mykiss* is known to be moderately intolerant of poor water conditions (intolerance rating of 3.4) and no impairment of health was evident in the examples captured. In this segment, the presence of an exotic species and not the degradation of health (e.g. water quality) is thus responsible for the absence of indigenous species. However, the FAII does not consider the influence of invasive alien species on the indigenous fish communities.

At site 3 on the Hout Bay River, the presence of an unexpected indigenous species, increased the rating of this site from Class C to Class A. However, at Site 3 and 4 on the Lourens River, the presence of an exotic species *T. sparrmanii*, together with the presence of indigenous species, did not influence the FAII score negatively as would be expected. This would not be a true reflection of the integrity of the site if the exotic species were to be dominant, although expected indigenous species are present. This again confirms that the negative impact(s) that exotic fish might have on indigenous species are not considered in the calculation of the FAII score.

It was noted that a definite change in habitat suitability occurred between autumn and spring, as would be expected of rivers located in the southwestern Cape. Because of this change, habitat suitability changes consequently, different species were observed at the same sites in different seasons. The preference of different species for different habitats is probably responsible for this phenomenon. This seasonal influence on the distribution and presence of fish is, however, not assessed by the FAII.

Since the rivers sampled are located in the southwestern Cape, which is known to have a low fish species richness, a maximum of two indigenous species were expected in these rivers. Due to this low expectance value, the loss of a single species would have a greater effect on the rating of the FAII score, than the case would be if more species were to be expected. Such a FAII score might thus not be a true reflection of the biological integrity of a specific segment. This is probably the greatest limitation of applying the FAII in the southwestern Cape, where a low species richness is the norm. The FAII is not considered to be suitable for the assessment of streams and rivers with a naturally low fish species richness (Kleynhans, 1999), as it is not highly responsive to a change in biological integrity involving a species richness lower than five (N. Kleynhans, *pers. comm.*).

*Recommendations to improve the application of the FAII in the southwestern Cape*

- The preference and intolerance ratings of different species, with regard to different variables, need to be ratified scientifically, although these ratings have been calculated by a panel of experts. This will require that FAII scores and ratings should be based on scientific data instead of subjective information as at present, which result in a false representation of the observed biological integrity of segments within a river. However, it might be impossible to perform such scientific studies on all possible fish species, in which case results obtained by the FAII would always have to be considered as a subjective representation of the actual biological integrity of the system.
- Due to river morphology and the site location, some habitats might not be suitable for sampling. It is thus possible that some species might not be observed and a false representation of the integrity of the site is reported. This stresses the need to include an assessability score to the different habitats of different sites. In addition, certain fish species are not easily sampled by the methods recognised by the FAII. There is thus a need to include a parameter indicating to what ease with which fish species are sampled by the different methods available. Since the FAII scores obtained are mostly already an under-estimation of the true biological integrity of a segment (Kleynhans, 1999), the above-mentioned limitations need to be included in order to prevent a further under-estimation of the integrity of a site.
- In this study, it is evident that the presence of invasive alien species might be responsible for an absence of indigenous species. Due to this absence of expected species, the integrity of the segment might be under-estimated. It is thus necessary to include intolerance ratings of indigenous species with regard to exotic species. Although this might be impossible to achieve, an alternative could be considered by including intolerance ratings of exotic species observed. These intolerance ratings of exotic fish species could be used as an indication of the quality of certain habitat components such as water quality (N. Kleynhans, *pers. comm.*). This would however, be indicative of the health of the specific segment in which the exotic species is observed, and not an indication of the integrity of the segment (N. Kleynhans, *pers. comm.*).
- The elevating effect due to the presence of an unexpected indigenous species on the FAII score, needs to be countered by a decrease in the FAII score when an exotic species is present. Currently, the co-occurrence of exotic and indigenous species, does not decrease the FAII score as would be expected, although the presence of exotic species is indicative of a

decrease in the integrity. However, the FAII is not responsive to this decrease due to the presence of aliens. The FAII needs to be adjusted accordingly.

- Seasonality seems to influence the habitat suitability of segments within rivers. Consequently, this change in habitat suitability influences the distribution of fish species during different seasons. Thus, the FAII needs to be adjusted to assess biological integrity according to different seasons, otherwise different results with regard to integrity will be obtained with a change in season. These will thus not be a true representation of the biological integrity observed.
- The rivers of the southwestern Cape are characterized by a low fish species richness. The absence of a single species will thus have a greater affect on the rating of the FAII score. This will inevitably result in a lower representation of the integrity observed. It is thus suggested that species richness and abundance should not influence the FAII score to the present extent. Table 3 thus needs to be adjusted according to the low species richness observed in the southwestern Cape.

### ***Conclusions***

Due to a natural low fish species richness in the rivers of the southwestern Cape, the use of fish as indicators of biological integrity needs to be re-considered. The observed low species richness, in addition to a lack of scientific information regarding expected fish species, indicates that fish are not suitable bio-indicators in the southwestern Cape. Since the use of the FAII is discouraged in areas with a low species richness (Kleynhans, 1999), the potential use of the FAII to investigate biological integrity in the southwestern Cape has to be re-considered or adjusted to incorporate conditions experienced in this area. It is suggested that the FAII only be used in general fish surveys to provide quantitative baseline data. Furthermore, it is recommended that specific fish species are used separately as bio-indicators of specific aquatic impacts and habitat characteristics, such as water quality and habitat suitability.

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## Chapter 3

### **Investigating estrogenic activity in the Lourens, Palmiet and Hout Bay Rivers and the production of vitellogenin in fish of the Lourens River**

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#### ***Introduction***

In recent years, increasing international concern has been raised about pollutants that are able to disrupt reproductive function by interacting with the endocrine system of biological organisms (Christen, 1998; Hecker *et al.*, 2002). Numerous studies investigated the effects of exposure to endocrine-modulation compounds in both humans and wildlife (Janssen *et al.*, 1995; Bayley *et al.*, 1999; Dalton, 2002). Most evidence for endocrine disruption (ED) in wildlife has come from organisms living in or closely associated with the aquatic environment (Jobling and Sumpter, 1993). In polluted aquatic environments, these organisms are constantly exposed to low concentrations of the pollutants (White *et al.*, 1994). An endocrine disrupter is defined as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or (sub)populations (Sole *et al.*, 2001). Estrogenic activity in aquatic systems may cause such adverse effects. Endocrine disrupters are released into aquatic systems in numerous ways: agricultural runoff, organic waste and ineffective waste-water treatment facilities (Eaton and Lydy, 2000). Results from various studies showed that both natural and synthetic components present in the environment are capable of disrupting normal endocrine function (Bayley *et al.*, 1999).

Bio-indicators are used to assess the health status of organisms and to obtain early-warning responses of environmental risks (Payne *et al.*, 1987). Fish, as bio-indicators for biological monitoring, have a high public profile and consequently are used extensively for conservation status and bioaccumulation studies. In aquatic ecosystems, fish or fish communities are regarded as indicators of overall system health (Barnhoorn, 2001). When using fish as bio-indicators one may avoid unacceptable and often irreversible effects such as mortality and loss of species.

When an aquatic system is polluted, fish can be affected directly or indirectly. The direct effects are initiated at the lower level of biological organisation (molecular or sub-cellular level). These effects of the pollutant propagate upwards through the levels of biological organisation and affect physiological and metabolic processes (Barnhoorn, 2001). Ultimately these can be manifested as changes at the population and community levels (Thomas, 1990), and may in the long term thus influence the biodiversity of a river.

The most important natural estrogen in vertebrates is  $17\beta$ -estradiol (Jobling and Sumpter, 1993). This steroid plays a role in the hormonal pathways involved in the regulation and development of gametes, sexual phenotype and sex-specific characteristics (Jobling and Sumpter, 1993). A wide range of chemicals has been found to mimic the action of  $17\beta$ -estradiol in fish and other vertebrates (Chambers, 1984). These xenoestrogens (endocrine disrupters) may cause dramatic ecophysiological responses, for example, the induction of detoxification enzymes, organ dysfunction, reduced fish community integrity and impaired reproduction and growth (Adams *et al.*, 1999), all of which are non-specific responses. Earlier studies linked polychlorinated biphenyls and some pesticides to reproductive problems in fish-eating birds and changes in fish reproductive organs (Christen, 1998). A decrease in the rate and intensity of male guppy sexual display, after exposure to natural estrogen ( $17\beta$ -estradiol) and the xenoestrogen (4-*tert*-octylphenol), has been recorded (Bayley *et al.*, 1999). Estrogenic compounds have also been found to cause a decrease in testosterone levels in male fish (Gunderson *et al.*, 2000), changes in gonad structure (Bayley *et al.*, 1999) and a large increase in the liver somatic index (LSI) (Donohoe *et al.*, 1999).

The potential threat to animal reproduction posed by the presence of xenoestrogens in the environment has been met by an intensive global effort to identify and develop biomarkers suitable for screening chemicals for their estrogen mimicking capacity. It is of the utmost importance to identify biomarkers that identify non-lethal effects of aquatic contamination. This foresees that negative effects due to contamination can be identified before changes in fish community structures (including death) and fish assemblages can occur. The biomarkers identified as being capable of measuring estrogen activity of xenoestrogens, have mostly been assessed during *in vitro* studies (Arnold *et al.*, 1997) or at cellular level (Janssen *et al.*, 1995). At a higher level of organisation, *in vivo* vitellogenin production in male fish has been measured (Donohoe *et al.*, 1999). These methods have proven highly sensitive to xenoestrogenic activity of chemicals (particularly in the case of vitellogenesis), and have been central in revealing the presence of these chemicals in the environment (Bayley *et al.*, 1999).

Vitellogenin (Vtg) production in male fish was shown to be a good biological indicator (specific response) of estrogenic activity (Monteverdi and Di Giulio, 1999) and is well documented. Vtg is a phospho-lipoglycoprotein synthesized by the liver of virtually all oviparous animals (Montverdi and Giulio, 1999). Production of Vtg (vitellogenesis) in the females of oviparous fish species occurs in response to increased concentrations of estrogens circulating in the blood. This is normally triggered by environmental stimuli such as increased water temperature and photoperiod associated with the reproductive season (Monteverdi and Giulio, 1999). Although livers of male fish are capable of producing Vtg, endogenous production of Vtg is female-specific, as male fish normally display plasma estrogen levels that are too low to elicit an appreciable vitellogenic response (Wallace, 1985).

The production of Vtg has, however, been detected in male fish from polluted environments. This suggests that xenoestrogens enter aquatic environments at concentrations high enough to cause ecological effects (Christen, 1998). These concentrations of estrogenic compounds, together with the fact that male fish potentially can produce Vtg, suggests that feminisation may take place after exposure to estrogenic substances. This may ultimately lead to changes in fish assemblages, since a high prevalence of the female phenotype (Metcalfé *et al.*, 2001), in addition to hermaphroditism (Sole *et al.*, 2001), have been observed in various aquatic systems. In addition, the exposure to xenoestrogens has shown to decrease Vtg levels in female fish (Donohoe *et al.*, 1999, Thomas, 1989). This clearly indicates that, in addition to Vtg production in male fish, the suppression of Vtg production in female fish may be a suitable indicator of reproductive dysfunction, because adequate yolk reserves must be available to support developing embryos (Donohoe *et al.*, 1999).

High concentrations of azinphos-methyl, chlorpyrifos and endosulfan ( $\alpha$ ,  $\beta$  and S), entering the river via agricultural runoff and spraydrift, have been detected in the Lourens River (Schulz, 2001). These chemicals may also enter rivers through urban runoff or as organic waste (Schulz *et al.*, 2001) and are known to cause endocrine disruption in freshwater fish (Kime, 1998). In addition, these chemicals are toxic to aquatic organisms, evidenced by fish kills and eggshell thinning in the African fish eagle (Grobler, 1994). Being exposed to possible agricultural runoff, the Palmiet and Hout Bay Rivers may also be contaminated by these agricultural and urban related chemicals, leading to endocrine disruption.

The main objectives of this study are 1) to investigate the cytotoxicity of water samples taken at each site from the Lourens, Palmiet and Hout Bay Rivers; to determine putative estrogen activity of these water samples; 3) to determine whether Vtg production occurs in wild caught male fish in the case of exposure to estrogenic substances in the Lourens River and 4) to determine possible other effects of estrogenic exposure in male fish of the Lourens River such as changes in gross gonad morphology.

It is expected that fish at segments downstream from agricultural activity and industrial and urban areas may demonstrate affects of exposure to estrogenic substances. These could include possible vitellogenin production in male fish, feminisation and abnormal gonad morphology. Fish upstream from these sites should not experience any of the effects of endocrine disruption mentioned.

## Materials and Methods

### Study area

Study material was collected from the the Hout Bay, Palmiet and the Lourens Rivers. These three rivers occur within the southwestern Cape and, because it occurs in a Winter Rainfall Region, have a lower fish species richness (Table 1) than rivers in the Summer Rainfall Region (Deacon and Kemper, 1999). Three segments in the Hout Bay River, four in the Lourens Rivers and five in the Palmiet River, were chosen as study sites (Table 2). These segments were chosen to be representative of the freshwater reaches in the rivers, and are characterized by different habitats and flow regimes.

**Table 1.** Abiotic characteristics of the four rivers to be studied. (\* = data not available at present).

River	Catchment Area (km <sup>2</sup> )	Length (km)	Mean annual rainfall (mm)	Mean annual runoff (m <sup>3</sup> )
Palmiet	465	74	1139	310 x 10 <sup>6</sup>
Hout Bay	33.8	12	1457	*
Lourens	92	20	915	21 x 10 <sup>6</sup>

**Table 2.** Location of the different sites per river sampled.

River	Site no	Location	Descriptive locality
Lourens	Site 1	34° 01.741' S; 18° 57.407' E	Lourensford Estate (IFR site 1)
	Site 2	34° 04.502' S; 18° 53.341' E	Vergelegen Estate (IFR site 2)
	Site 3	34° 04.983' S; 18° 32.144' E	Radloff Park (IFR site 3)
	Site 4	34° 05.775' S; 18° 49.772' E	Victoria Road, Strand (IFR site 5)
Palmiet	Site 1	34° 03.351' S; 19° 02.465' E	Nuweberg Forest (IFR site 1)
	Site 2	34° 07.102' S; 19° 01.481' E	Grabouw town centre
	Site 3	34° 14.641' S; 18° 59.666' E	Arieskraal Farm (IFR site 2)
	Site 4	34° 17.185' S; 18° 58.701' E	Kogelberg Nature Reserve (IFR site 3)
	Site 5	34° 19.241' S; 18° 58.170' E	Kogelberg Nature Reserve (IFR site 4)
Hout Bay	Site 1	33° 58.449' S; 18° 24.601' E	Upstream from Hely Hutchinson Dam
	Site 2	34° 00.931' S; 18° 22.863' E	Disa Road Bridge, Hout Bay
	Site 3	34° 01.758' S; 18° 21.231' E	Victoria Road Bridge, Hout Bay

Although all three rivers experience agricultural runoff (including potential pollutants such as pesticides, herbicides and fertilizers) and dumping of sewage and industrial waste products, Site 1 in all three rivers was chosen upstream of most human or other potential negative impacts. The rivers, and more specifically all the different sites within the rivers are thus expected to experience various degrees of disturbance, with Site 1 serving as a potential pristine control site.

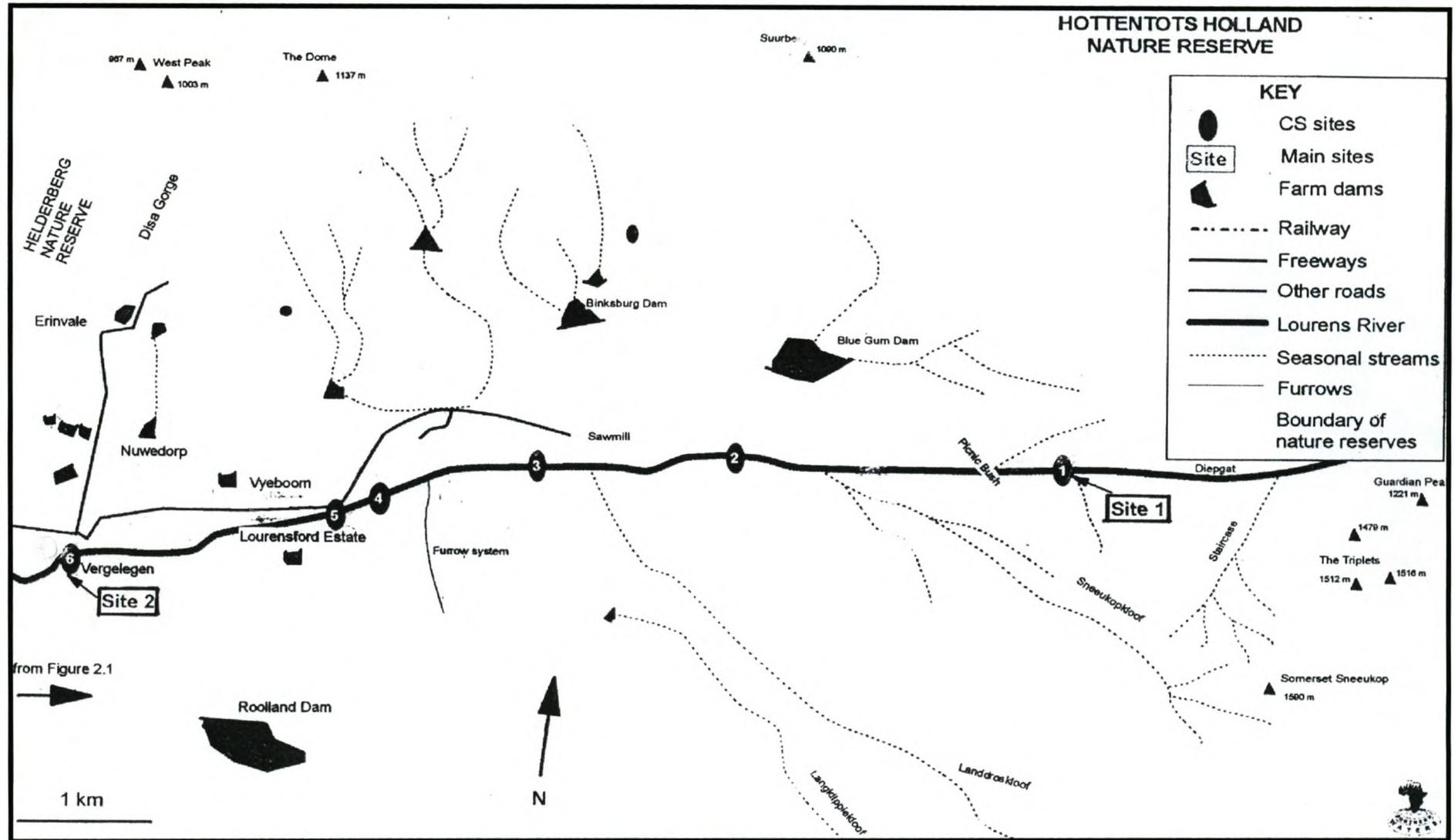
Fish collected from the Lourens River was used to study the incidence of estrogenic activity in male fish and histo-pathological effects in the gonads of fish. The Lourens River has its source in the Helderberg Mountains and its estuary situated at the Strand. Four segments, characterized by different habitats and flow regimes, were chosen as sampling sites (Figure 1). All the sites, except Site 1, experience various degrees of agricultural runoff, residential stormwater runoff and dumping (Table 3). Sites 2–4 are thus expected to experience various degrees of pollution.

**Table 3.** Major landuses at the four sites and possible impacts affecting water quality

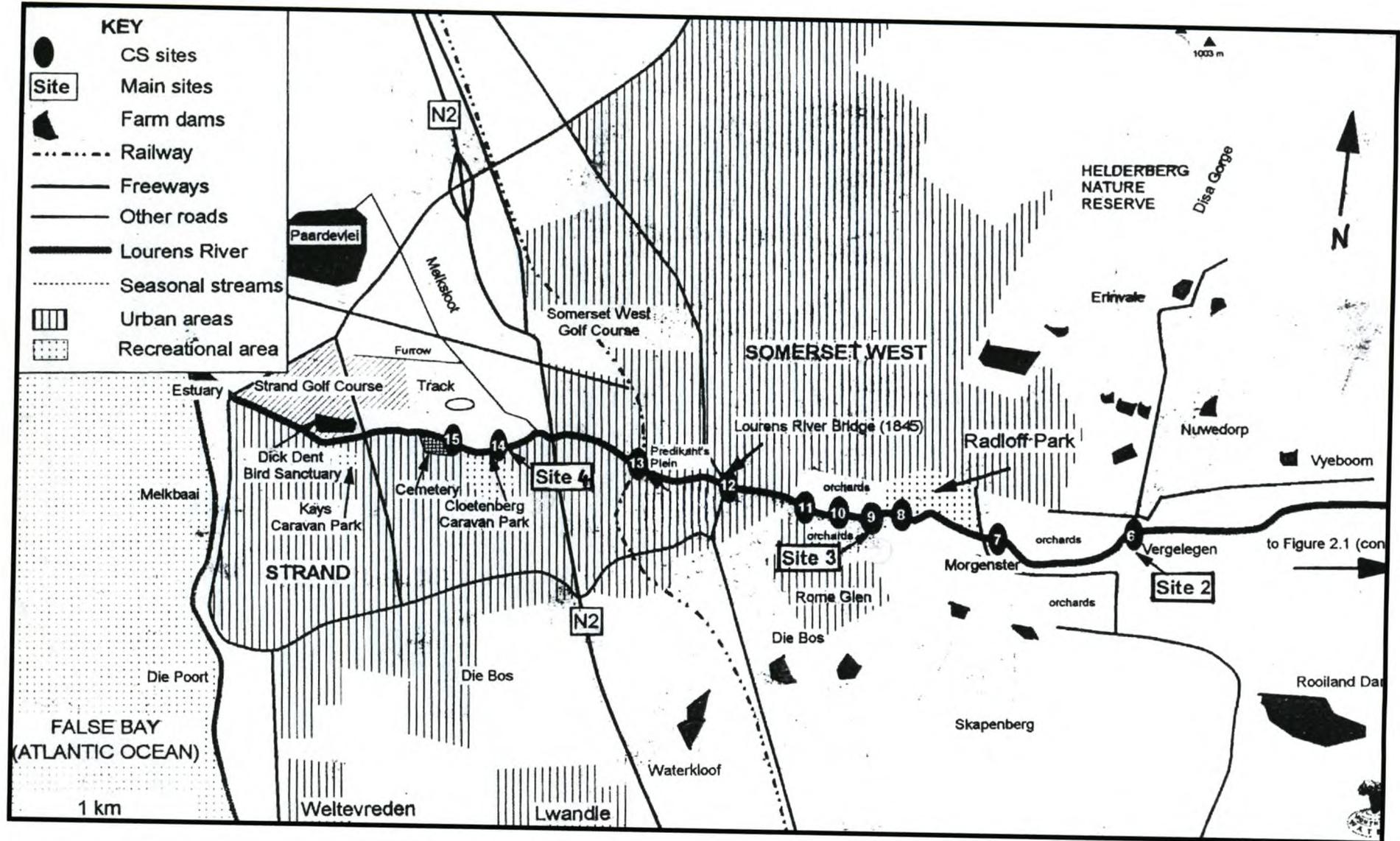
Site	Zone	Major landuse	Possible impacts
1	Mountain stream	Natural fynbos area	Alien plant invasions, hikers and occasional vehicles
2	Foothill river	Agriculture, including orchards, livestock and a nursery	Agricultural runoff, afforestation, sawmill, an upstream weir and stormwater runoff
3	Foothill river	Residential, agriculture and recreation	Urban runoff, stormwater runoff, deposition of rubble, channel modification, agriculture and erosion
4	Coastal plain river	Agriculture, residential	Industrial, agricultural, stormwater and urban runoff

### *Water sampling*

Water samples were collected in 2002 during autumn (April) and spring (September) at all sites (Table 2) of the Lourens, Palmiet and Hout Bay Rivers. Water samples were collected in 500 ml glass bottles with aluminium lids and stored at 4 °C for not longer than 48 hours before extraction. For water to be screened for estrogenic activity by *in vitro* bioassay (tissue culture), hydrophobic compounds were extracted using Isolute SPE 6 ml C-18 solid phase columns (Anachem, South



**Figure 1.** Study area of the Lourens River, indicating the location of the four study sites and the fifteen Conservation Status (CS) sites (Tharme *et al.*, 1997).



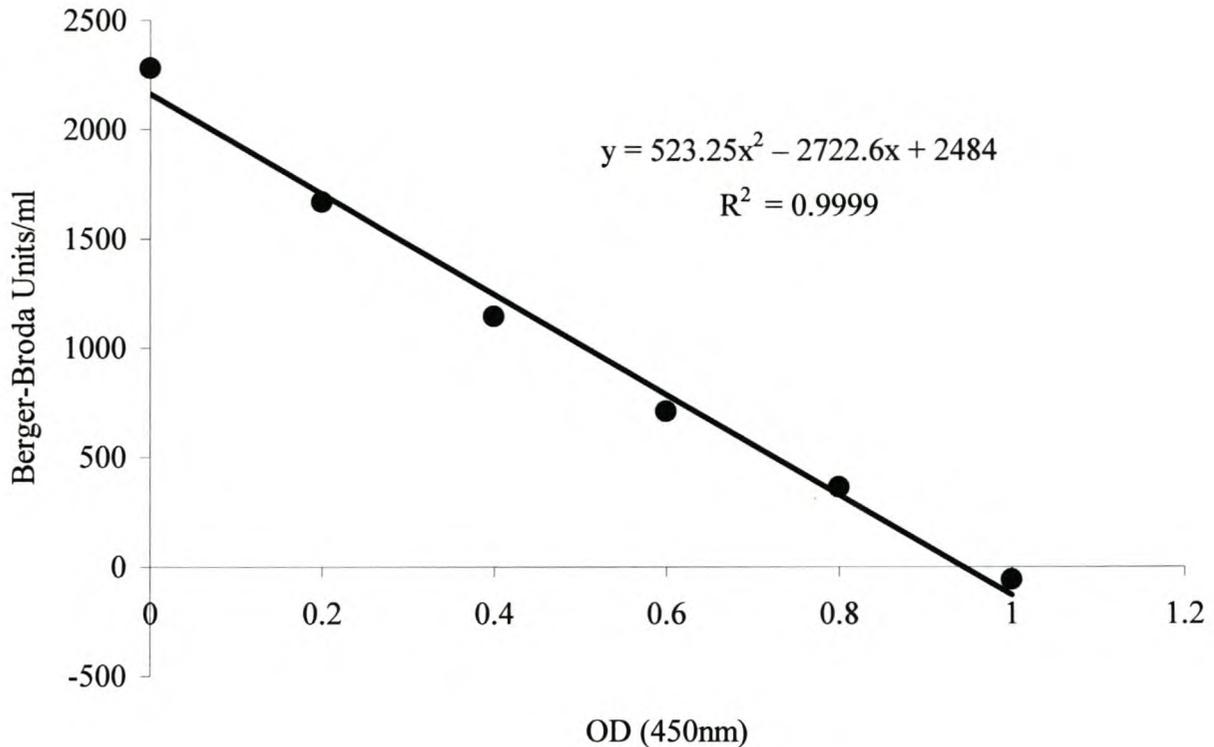
**Figure 1 (cont.)** Study area of the Lourens River, indicating the location of the four study sites and the fifteen Conservation Status (CS) sites (Tharme *et al.*, 1997).

Africa). Hydrophobic substances, bound to solid phase, were eluted with an eluent mixture containing 40 % hexane, 45 % methanol and 15 % 2-propanol. The eluents were collected in glass tubes, air dried and re-dissolved in ethanol to a final concentration of 1/100 of that of the original volume.

#### *General cytotoxicity of water samples*

Basic cytotoxicity screening of water samples was done by taking aliquots of the un-extracted water samples and incubating it in human whole blood culture (Bergmeyer and Bernt, 1974). Lactate dehydrogenase (LDH) leakage from the cells was used as a biomarker for cytotoxicity. Toxins or otherwise toxic compounds associated with cell damage will result in leakage of intracellular material into the surrounding extracellular environment. LDH increase in the surrounding medium was therefore used as an indicator of such damage.

For this assay, normal physiological saline solution was used as a negative control and physiological saline contamination with detergent (0.1 % v/v) as toxic positive control. Cytotoxicity of the water samples was evaluated by comparing LDH levels in the culture medium following the incubation period, to the values obtained with the negative and positive control samples. Samples were assayed in duplicate using a validated LDH procedure according to Bergmeyer and Bernt (1974). The optical densities were measured with a Labsystems Multiscan MS spectrophotometric plate reader at 450 nm. When employing tissue culture screening bioassays, cytotoxicity testing is necessary to ensure that false negative testing is excluded. Results are expressed in terms of Berger-Broda Units per ml (B-B units/ml) and read directly from the standard curve.



**Figure 2.** Standard calibration curve for LDH activity (B-B Units/ml).

### ***Estrogenic activity in water samples***

*In vitro* culturing of the water sample extracts with slices of *Xenopus* liver to study estrogenic stimulation of Vtg production was performed as described by Hurter *et al.* (2002). All procedures were carried out aseptically in a laminar flow cabinet. In brief, slices of *Xenopus laevis* liver tissue, measuring approx. 1 mm<sup>3</sup>, were prepared and placed in culture plates (Nalge Nunc, Denmark) and covered with 400 µl medium. The medium used for tissue culture was RPMI 1640 containing L-glutamine, NaHCO<sub>3</sub> and 25 mM Hepes (Highveld Biologicals, South Africa). A mixture of penicillin, streptomycin and fungizone (Highveld Biologicals) was added to the RPMI medium according to the manufacturer's instructions. Four replicate cultures were prepared for each water sample tested. Control samples were included on every plate. Negative controls contained medium and ethanol only, while positive controls contained medium with 0.1 ppm 17-β-estradiol (Sigma Chemical, Missouri) and ethanol. The cultures were incubated in the dark at 27 °C. Culture media were replaced with fresh medium after 3 days in all culture wells. Medium collected after Day 6 were used for vitellogenin analysis.

The medium was aspirated and stored at  $-80\text{ }^{\circ}\text{C}$  for vitellogenin determination using a frog, *X. laevis*, Vtg ELISA (Pool *et al.*, 2002). The optical densities were measured with a Labsystems Multiscan MSTM plate reader at 450 nm. Vitellogenin concentrations were calculated from a standard curve prepared using samples of known vitellogenin concentration on each plate, and compared to the negative control.

#### *Fish collection*

Fish were collected from the Lourens River in autumn (April) and spring (September) by means of electro fishing and a large seine net (3 m x 1.5 m). Fish species collected included *Tilapia sparmanii* (Banded tilapia), *Sandelia capensis* (Cape Kurper), *Galaxias zebratus* (Cape galaxies) and *Onchorhynchus mykiss* (Rainbow trout). As *T. sparmanii* and *S. capensis* were most frequently collected, these species were chosen as test organisms. A total of twenty eight fish from different sites (Table 3) were kept separately in clean glass tanks for not longer than 48 hours before blood samples were collected.

#### *Blood and tissue collection*

Blood was collected from the caudal vein using 1.1 – 1.2 mm heparinized capillary tubes. Following centrifugation for 4 min at 1.500 g, plasma was removed and frozen at  $-4\text{ }^{\circ}\text{C}$  for future analyses. Fish specimens were fixed in 4 % formalin and preserved in 70 % ethanol for future dissection.

#### *Presence of Vtg in plasma from male fish*

Blood plasma from *S. capensis* and *T. sparmanii* was used for Vtg determinations by using SDS-PAGE gel electrophoresis (Hurter *et al.*, 2002). In brief, 2  $\mu\text{l}$  bromo-phenol blue and 46  $\mu\text{l}$  treatment buffer were added to 2  $\mu\text{l}$  of plasma. Samples (10  $\mu\text{l}$ ) were applied to the wells and the gel was run at a constant current of 20 mA. Gels were stained with 0.125% (w/v) Coomassie Blue in 50% methanol and 10 % (v/v) acetic acid. This solution was loaded on a 7.5 % monomer SDS-PAGE gel. In addition to a positive and negative control, a high-molecular-weight range protein molecular weight marker (AEC-Amersham, South Africa) was included on each gel (Figure 3).

Positive and negative control samples were obtained from a *T. sparmanii* individual. Such control samples from *S. capensis* was unavailable due to insufficient numbers collected of the species. The

*Statistical analyses*

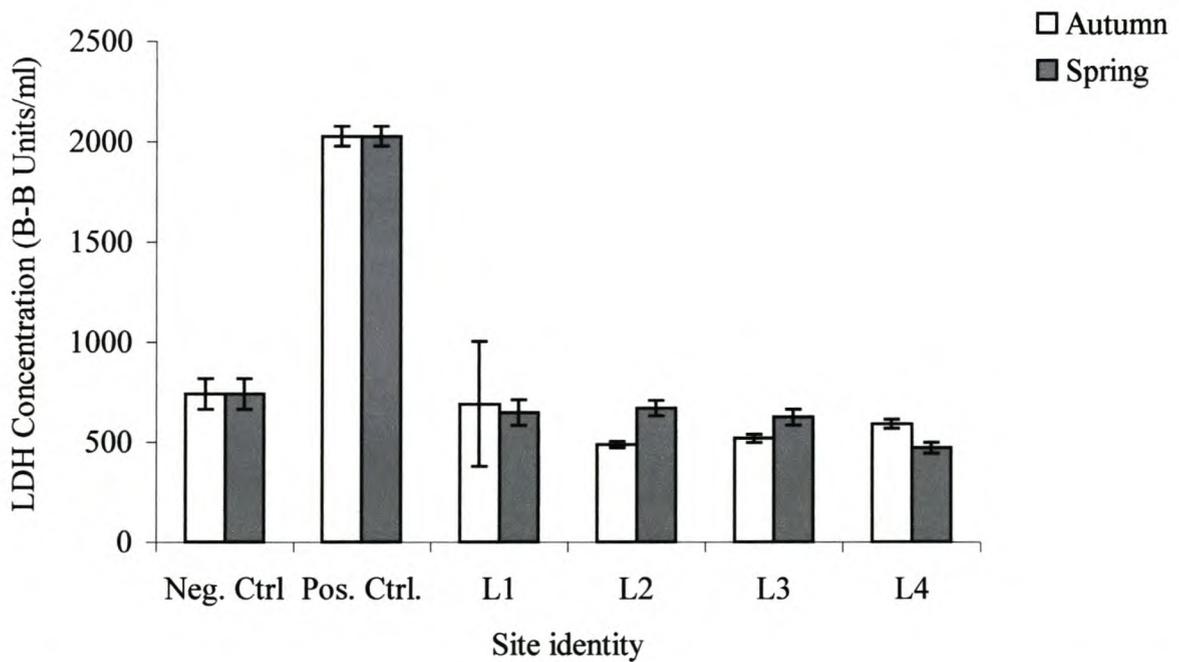
Means and standard deviation were calculated for all replicates per sample site. Water samples were recognised as cytotoxic or estrogenic if the observed LDH or Vtg values were significantly different ( $P < 0.001$ ) from the negative control. Statistically significant differences ( $P < 0.001$ ) were confirmed by One-Way Analysis of Variance (ANOVA) and Multiple Comparison testing (groups vs control comparisons), Dunnet's Method. All analyses were performed using the software package SigmaStat 1.0.

## Results

### *Cytotoxicity of water samples*

#### Lourens River

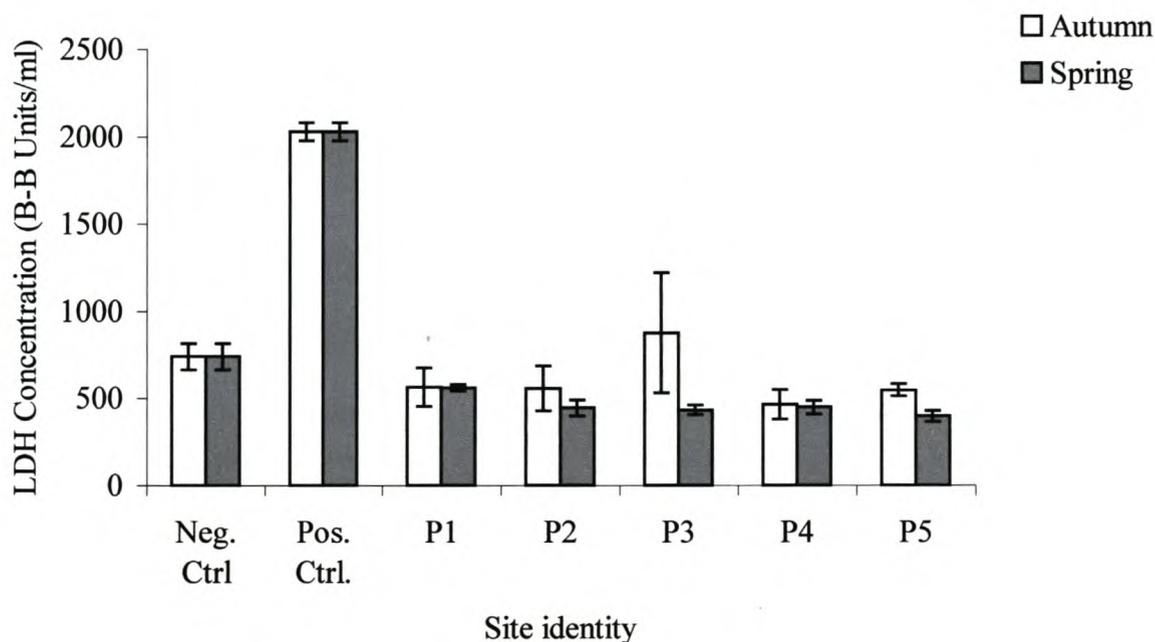
The results obtained indicate that only the toxic positive control resulted in higher LDH activity in the medium (Figure 4). No significant difference ( $P > 0.05$ ) was found between the samples and the negative control. A significant difference (ANOVA;  $F = 32.1$ ,  $P < 0.05$ ) was found between all the samples and the positive control. Variation between samples from the same sites from different seasons were observed. However, this cytotoxicity did not differ significantly ( $P > 0.05$ ) from the negative control.



**Figure 4.** Histogram to indicate the cytotoxicity and standard deviations of samples from the Lourens River in comparison to the negative control and the toxic positive control. Grey bars indicate samples taken in spring and white bars samples taken in autumn. Numbers indicate the different sites. No significant difference ( $P < 0.05$ ) was found between the samples and the negative control. A significant difference ( $P < 0.05$ ) was found between all the samples and the positive control.

### Palmiet River

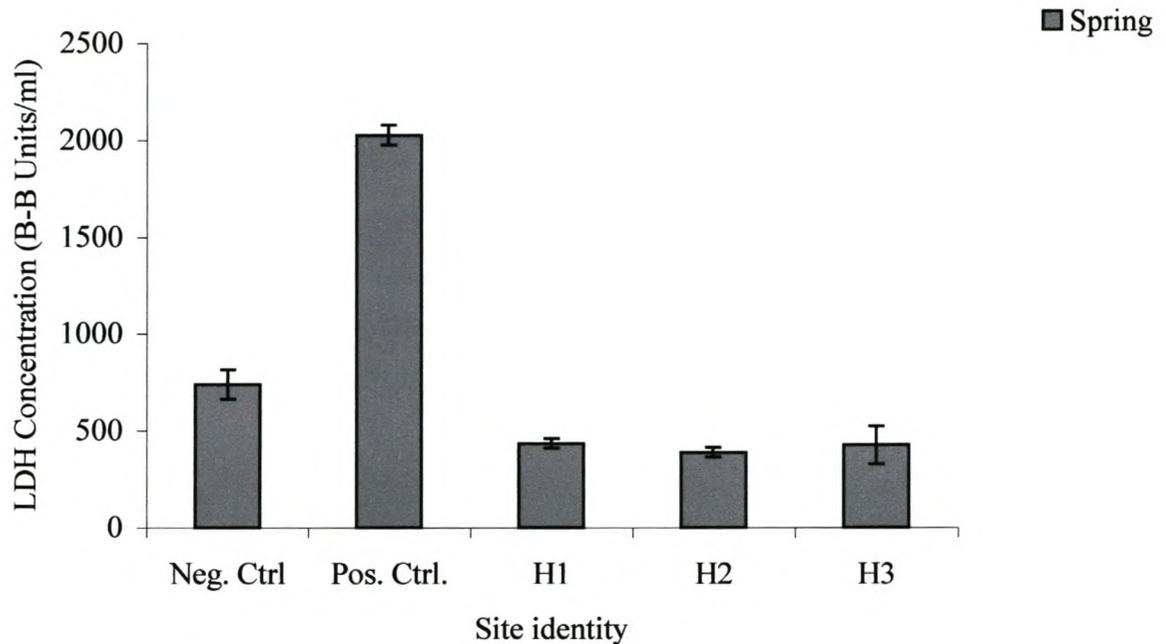
The results obtained indicate that only the toxic positive control resulted in higher LDH activity in the medium (Figure 5). No significant difference ( $P > 0.05$ ) was found between the samples and the negative control. A significant difference (ANOVA;  $F = 32.1$ ,  $P < 0.05$ ) was found between all the samples and the positive control. Variation between samples from the same sites from different seasons were observed. However, this cytotoxicity did not differ significantly ( $P > 0.05$ ) from the negative control.



**Figure 5.** Histogram to indicate the cytotoxicity and standard deviations of samples from the Palmiet River in comparison to the negative control and the toxic positive control. Grey bars indicate samples taken in spring and white bars samples taken in autumn. Numbers indicate the different sites. No significant difference ( $P > 0.05$ ) was found between the samples and the negative control. A significant difference ( $P < 0.05$ ) was found between all the samples and the positive control.

### Hout Bay River

The results obtained indicate that only the toxic positive control resulted in higher LDH activity in the medium (Figure 6). No significant difference ( $P > 0.05$ ) was found between the samples and the negative control. A significant difference (ANOVA;  $F = 32.1$ ,  $P < 0.05$ ) was found between all the samples and the positive control.



**Figure 6.** Histogram to indicate the cytotoxicity and standard deviations of samples from the Hout Bay River in comparison to the negative control and the toxic positive control. Numbers indicate the different sites. No significant difference ( $P > 0.05$ ) was found between the samples and the negative control. A significant difference ( $P < 0.05$ ) was found between all the samples and the positive control.

*Estrogenic activity of water samples*

No significant production of Vtg was observed in any of the water samples from the Lourens, Palmiet and Hout Bay Rivers (Table 4). The possibility of reporting false negatives seems unlikely since the positive controls generated the expected response in this assay. Results from this study are seen as qualitative since Vtg production in the water samples are compared to Vtg production in the negative control. Results are thus indicative of an increase in Vtg and the multiple replicates did not differ significantly from the negative control.

**Table 4.** Estrogenic activity in water samples using an *in vitro* *Xenopus laevis* liver culture bioassay. A ‘-’ indicates samples with no significant ( $p > 0.001$ ) estrogenic activity.

River	Spring sample					Autumn sample				
	1	2	3	4	5	1	2	3	4	5
Lourens	-	-	-	-	-	-	-	-	-	-
Palmiet	-	-	-	-	-	-	-	-	-	-
Hout Bay	-	-	-							

*Detection of Vtg in fish plasma*

In 60% (N = 15) of male *T. sparmanii* and *S. capensis*, the presence of Vtg was detected. This is evident from visible bands that correspond to the Vtg proteins showed in the positive control specimen (Figure 7). As expected, Vtg was detected in the plasma of all the females (N = 13) from all the sites.

## ***Discussion***

Results from this study indicate that 60 % of male fish from the Lourens River produce the Vtg protein. However, no obvious abnormal gonad development was found in male or female fish from this river. Furthermore, results indicate that the water samples collected from the Lourens, Palmiet and Hout Bay Rivers were all negative with regard to cytotoxicity and estrogenic activity.

The possibility of reporting false negative results with regard to the cytotoxicity and estrogenicity of water samples is unlikely since the positive controls generated the expected response in both bioassays. However, these results must be viewed with great caution against the evidence on a worldwide scale, that endocrine disruption has been recognized as a top priority focus area in the field of ecotoxicology. This intense interest becomes understandable when one considers the wildlife case studies, including the demasculisation of certain alligator populations (Guillette *et al.*, 1994), or the feminisation of some fish species due to exposure to estrogenic hormones and the mimics in sewage, industrial and agricultural runoff.

Furthermore, results from this study have to be viewed with caution since water sampling was a once-off event and only represents the immediate or present state of the rivers. More frequent sampling would be necessary to exclude the possibility that estrogens might be present in the rivers studied, but not detected as a result of seasonal flux. After all, in the context of the Western Cape, the most important period with regard to agricultural runoff is when the first heavy rains fall at the beginning of winter (Schulz *et al.*, 2001).

Runoff is regarded as an important route of entry of nonpoint source pollutants in surface waters in arid areas (Everts, 1997). Following runoff events, high concentrations of chemicals that may cause endocrine disruption have been detected in the Lourens River (Schulz *et al.*, 2001). These chemicals include azinphos-methyl, chlorpyrifos and endosulfan ( $\alpha$ ,  $\beta$  and S) of which concentrations exceeded the target water quality range proposed by the Department of Water Affairs and Forestry (DWAF).

Contaminants which may cause endocrine disruption may also be present in the Palmiet and Hout Bay Rivers, since they are exposed to the same putative human impacts (agriculture and urbanization) as the Lourens River. Certain water chemistry constituents of various sites of the Palmiet River were found to be outside the expected natural range for equivalent unimpacted sites

(Brown and Day, 1998). However, information regarding levels of contamination in these rivers is lacking. A study focussing on the physical and chemical water quality of these rivers was done in parallel with this study but results are not yet available.

It is also possible that contaminants in water samples are not detected but through bioaccumulation, may result in endocrine disruption in aquatic organisms. Furthermore, since many pesticides and herbicides are hydrophobic, sediments may act as a sink for aquatic contaminants. Contaminants may re-enter the water column as a result of wave-action, which may cause physical re-suspension (Schulz, 2001). Concentrations of pesticides and herbicides in sediments have been found to exceed concentrations observed in the water (Schulz, 2001). Future research should also focus on investigating the estrogenic activity of sediments.

The production of vitellogenin in male fish from the Lourens River, support the possibility of estrogenic activity in water samples: seasonal flux might influence levels of contaminants, contaminants are bioaccumulated over time and are thus not detected in water samples and that sediments may contain high concentrations of contaminants. Vitellogenin production and abnormal gonad morphology in male fish have proven to be indicative of estrogenic activity (Kime, 1998; Folmar *et al.*, 2001). Abnormal gonad morphology, which was not found in the present study, will affect reproductive success, as a decrease in spermatogenesis will decrease fertilization success. However, little is known regarding the effects of Vtg production on fish populations. The required concentration and the exposure period to endocrine disruptors that will cause Vtg production and abnormal gonad morphology are still unknown. Although the production of Vtg is indicative of estrogens present, phyto-estrogens (plant hormones) cannot be discarded (Kime, 1998). Furthermore, as Vtg is lipophilic and may accumulate maternally through bioaccumulation (Dauberschmidt and Hoffmann, 2001), it may be passed on to future generations. This stresses the fact that these physiological changes in fish could be the result of long-term exposure to endocrine disruptors.

As the Vtg protein was found in the plasma of male fish from the control site, the upstream migration of fish from downstream sites must be considered. However, insufficient information regarding migration patterns and the general biology of certain freshwater fish species exist, and have to be assessed during future research.

Future research should thus include more frequent water and sediment sampling. In addition, future studies should also focus on the dosage and exposure period needed to cause physiological changes, characterising endocrine disruption, migration patterns and the general biology of freshwater fish. More detailed research is also needed into the possible chemical contamination of fish (Kime, 1998) and reproduction rates of fish species.

Studies focusing on the biodiversity of ecosystems (Chapter 1) indicate the presence and survival of organisms. These studies are not highly responsive to subtle physiological changes, like endocrine disruption, which may be manifested as changes at population or community level (Thomas, 1989). Results from this study indicate that although male fish are present and appear healthy, they show signs of estrogenic activity.

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## Chapter 4

### Conclusions

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Due to a naturally low indigenous fish species richness, it is recommended that the use of the Fish Assemblage Integrity Index (FAII) as an indicator of river health in rivers of the southwestern Cape needs to be re-evaluated (Chapter 2). The use of the FAII to assess indigenous fish population integrity, as proposed by Kleynhans (1999), needs to be re-considered, as it does not seem to be highly responsive to a change in biological integrity. Furthermore, the FAII is not sensitive enough to certain impacts that may strongly affect the presence of indigenous fish species. These impacts include changes in habitat suitability and the influence of exotic alien fish species. This stresses the need for the incorporation of a habitat suitability score and a score representing the resistance of indigenous fish to the presence of exotic species. An upgraded edition of the FAII, which includes a habitat suitability score, is already being developed, but is not yet available (N. Kleynhans, *pers. comm.*). The FAII may be a useful mechanism to assess the presence of exotic alien fish species, if the general biology of both indigenous and exotic species is considered. However, the FAII is not responsive to the health of fish present as indicated by the results obtained in Chapter 3 regarding endocrine disruption. It is recommended that the FAII only be used to provide baseline data for fish surveys, rather than assessing river health. Furthermore, it is recommended that specific fish species be used as indicators of specific impacts that may affect river health. For example, *Onchorhynchus mykiss* has been shown to be an indicator of water quality, including endocrine disruption, and of the presence of heavy metals in aquatic systems (Skelton, 1993; Kleynhans, 1999). This species has an impact on biodiversity so this also needs to be weighed up carefully.

Vitellogenin (Vtg) production and abnormal gonad morphology in male fish have been employed as biomarkers for endocrine modulations and reproductive health (Kime, 1998; Folmar *et al.*, 2001). However, little is known regarding the effects of these physiological changes on fish populations. Abnormal gonad morphology will affect reproductive success, as a decrease in spermatogenesis will decrease fertilization success. However, the required concentration and exposure period to endocrine disruptors, which will affect gonad morphology, still needs to be studied. Endocrine disruption is known to adversely affect the growth, metabolism and reproduction of fish (Kime, 1998; Van der Kraak *et al.*, 2001; Knorr and Braunbeck, 2002), the effect of Vtg production on

male fish is still unclear. Although the production of this protein is indicative of the presence of estrogen, phyto-estrogens (plant hormones), anti-androgens (fungicides), and anti-estrogens cannot be discarded. Furthermore, as Vtg is lipophilic and may accumulate maternally through bioaccumulation (Dauberschmidt and Hoffmann, 2001), it may be passed on to future generations. This emphasizes the fact that these physiological changes in fish are the result of long-term exposure to endocrine disruptors and other toxicants. It is recommended that a range of biomarkers be used to anticipate possible effects of endocrine disruption. Furthermore, research should be done on the effects of endocrine disruption on female fish.

Contradictory to physiological changes in fish, the *in vitro* screening of water samples to detect endocrine disruption (Chapter 3) reveals the estrogen activity at the time of sampling. These studies may be used as a preliminary screen for estrogen activity to determine hotspots or problem areas. As indicated in Chapter 3, these studies may give false negative results, as fish themselves may be estrogenic. In addition, estrogens in the water may not yet be bio-available to the target organs of fish for endocrine disruption to occur. As previously mentioned, the concentrations of estrogen, and the exposure period needed for physiological changes to take place are still unknown. This stresses the need for future research to focus on the dosage and exposure period needed to cause physiological endocrine disruption.

Overall, fish have a potential as bio-indicators of river health. However, it is likely that a combination of various biotic (exotic alien fish) and abiotic factors (water quality) affects the integrity of indigenous fish populations. All of these putative impacts need to be considered and assessed before fish can be regarded as indicators of river health. In its present state, the FAII cannot be applied to assess the health and integrity of indigenous fish populations in the southwestern Cape. Physiological studies focussing on fish health may be used to determine subtle non-lethal effects, which may affect the health of fish populations in the long term. Rapid *in vitro* screens for endocrine disruptors and other toxicants may also be used if factors like seasonality are considered.

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