

ABSTRACT

Title: A qualitative and quantitative assessment of the normal histology of selected target organs of *Clarias gariepinus* and *Oreochromis mossambicus*

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A histological assessment allows aquatic scientists to assess fish health in polluted aquatic ecosystems at tissue and cellular level. However, a firm knowledge and understanding of normal histological structure is essential to ensure accurate and objective results. Hence, the histologist must be able to distinguish between toxicant induced lesions and the range of histological characteristics considered to be normal for that specific species. However, limited histological reference material and data describing normal conditions are available for southern African fish species.

The aim of this baseline study was to establish reference material, both qualitative and quantitative, for two southern African freshwater fish species used as indicator species in toxicity studies at the University of Johannesburg, *Clarias gariepinus* and *Oreochromis mossambicus*. The reference material includes descriptive histology of selected target organs, and the associated values and intervals for related quantitative health aspects including somatic indices, condition factor, blood parameters (haematocrit and plasma proteins) and quantitative histological results. Essentially, this project originated from a need to better understand the normal histology of *C. gariepinus* and *O. mossambicus*, as a range of histological characteristics were identified in control groups in previous

toxicity studies which could not be confirmed to be associated with normal conditions.

To be able to conduct a baseline study, with the purpose of establishing reference material, it was essential that the history of the specimens be known and documented including age, sexual maturity, nutritional status and physical and chemical water quality data. The project therefore involved the breeding of fish specimens under controlled conditions. A subsequent necropsy and qualitative and quantitative histological assessments were executed on five target organs (usually included in toxicity studies within the department): liver, gills, gonads, heart and kidney.

Fish of both species were bred in an environmental room in reconstituted, reverse osmosis water and reared until sexually mature. Twenty specimens with an approximately 50:50% sex ratio were collected for both species. A necropsy was performed on each specimen and tissue samples of the selected target organs were processed using standard techniques, and prepared for the qualitative and quantitative histological assessments using light microscopy.

The results showed that no macroscopic abnormalities were identified during the necropsy regarding external features or internal organs of the specimens within the sample groups, and all fish appeared to be in good health. The target organs were then examined microscopically, followed by a description of the normal histological structure (qualitative assessment). In addition, the target organs were assessed by means of a specific quantitative histological assessment protocol, which provides a standardised methodology of criteria to objectively assess fish health (quantitative assessment). Histological alterations identified during this assessment were quantified and subsequent reference index values could be calculated for each organ.

It is acknowledged that the confirmation of reference values and intervals representing normal conditions in fish requires the collaboration of the results of various qualitative and quantitative baseline studies, as different factors can influence the histological integrity of a fish specimen including age, season, sex, sexual maturity, nutrition, physical and chemical water quality and/or toxicant exposure. A single study cannot incorporate all the possible different influences these variables will have on the histological structure of target organs.

This project was therefore a first step in examining and understanding the normal histology of the two selected freshwater fish species. So, as part of ongoing research at the University of Johannesburg, to establish and advance fish histology in toxicity and aquatic health related studies in South Africa, the results of this project provide: **(1)** knowledge and a better understanding of freshwater fish histology of selected target organs of two indicator species; **(2)** contributes to a limited database regarding normal fish histology of species endemic to South Africa; **(3)** provides reference material for target organ histopathology with the application of a standardised assessment protocol, including specific criteria which will ensure proper and more accurate histopathological diagnoses; **(4)** will eventually contribute towards the creation of a comprehensive freshwater fish histology atlas for southern African species; **(5)** provides baseline values and intervals for quantitative parameters measured as part of histological assessments (somatic indices, selected blood parameters and condition factor); **(6)** and provides a working document, to be used in, and supplemented by the results of, future histological assessments, to assist in basic fish histology training within the Department of Zoology at the University of Johannesburg.

UITTREKSEL

Titel: 'n Kwalitatiewe en kwantitatiewe assessering van die normale histologie van geselekteerde teikenorgane van *Clarias gariepinus* en *Oreochromis mossambicus*

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'n Histologiese ondersoek stel akwatiese wetenskaplikes in staat om die gesondheid van vis in besoedelde akwatiese ekosisteme op sellulêre vlak te assesser. Vir dié doel, is dit nodig om voldoende kennis van die normale histologiese struktuur van die spesifieke visspesie te hê om akkurate resultate te verseker, en 'n objektiewe assessering te kan uitvoer. Die histoloog moet dus in staat wees om te kan onderskei of histologiese veranderinge die gevolg is van blootstelling aan toksiese stowwe, en of dit as normaal beskou kan word. Beperkte hoeveelheid verwysingsmateriaal wat die normale histologie van inheemse Suid-Afrikaanse visspesies beskryf, is beskikbaar.

Die doel van hierdie studie was om verwysingsmateriaal, soos hierbo genoem, te verskaf. Dit sluit in beide kwalitatiewe, en kwantitatiewe resultate vir twee Suider-Afrikaanse visspesies wat as indikatorspesies in toksisiteitstudies by die Universiteit van Johannesburg gebruik word, naamlik *Clarias gariepinus* en *Oreochromis mossambicus*. Die verwysingsmateriaal sluit die beskrywende histologie van spesifieke teikenorgane in, asook die kwantitatiewe waardes en intervalle vir somatiese indekse, die kondisiefaktor, sekere bloedparameters (hematokrit and plasma proteïen bepaling) en kwantitatiewe histologiese resultate. Die studie is oorspronklik beplan omdat daar 'n behoefte ontstaan het om die normale histologie van *C. gariepinus* en *O. mossambicus* beter te

verstaan, nadat histologiese veranderinge in kontrole groepe in vorige studies in die departement geïdentifiseer is, wat nie as normal bevestig kon word nie.

Om 'n basislynstudie uit te voer met die doel om verwysingsmateriaal daar te stel, was dit belangrik om die geskiedenis van die vis te dokumenteer. Dit sluit die ouderdom, geslagsrypheid, voedingstoestand asook die nodige fisiese en chemiese waterkwaliteit analise in. Vir dié doel is die vis onder gekontroleerde toestande gebroei. Vervolgens is 'n nekropsie en 'n kwalitatiewe en kwantitatiewe assessering uitgevoer op vyf spesifieke teikenorgane. Hierdie teikenorgane word normaalweg in toksisiteitstudies gebruik en sluit die lewer, kiewe, voorplantingsorgane, hart en niere in.

Vis van beide spesies is uitgebroei in hersaamgestelde water wat eers gefiltreer is deur middel van 'n omgekeerde osmose proses. Die broeiproses is uitgevoer in 'n omgewingskamer en die vis is gemonitor totdat gelslagsrypheid bereik is. 'n Groep van twintig vis met 'n geslagsverskil van min of meer 50:50% is vir elke spesie gekies. 'n Nekropsie is op elke individu uitgevoer en weefselmonsters van die spesifieke teikenorgane is versamel. Die weefselmonsters is voorberei vir ligmikroskopiese analise deur die uitvoer van standaard tegnieke.

Tydens die nekropsie is geen makroskopiese abnormaliteite waargeneem met betrekking tot die uitwendige voorkoms of die inwendige organe van enige van die vis nie, en alle vis was in 'n goeie gesondheidstoestand. Die teikenorgane is vervolgens mikroskopies geassesseer, en die normale histologiese struktuur is beskryf met behulp van geannoteerde mikrograwe. Elke teikenorgaan is ook deur middel van 'n kwantitatiewe histologiese assesseringsmetode geëvalueer. Hierdie standaard metode stel kriteria daar om visgesondheid objektief te assesser. Histologiese veranderinge wat geïdentifiseer is tydens die kwantitatiewe assessering, is verwerk, en indekswaardes is bereken vir elke orgaan.

Resultate van verskeie basislynstudies is nodig om die waardes en intervale van verwysingsmateriaal te bevestig. Dit is nodig aangesien verskillende faktore die histologiese struktuur van 'n orgaan kan verander, naamlik ouderdom, seisoen, geslag, geslagsrypheid, voeding, fisiese en chemiese waterkwaliteit en blootstelling aan toksiese stowwe. Die effek van al die veranderinge kan nie in 'n enkele studie bestudeer word nie.

Hierdie studie word dus beskou as 'n eerste stap om die normale histologie van die twee visspesies te bestudeer en te verstaan. Die resultate van hierdie studie dra dus by tot die histologiese navorsing wat huidiglik uitgevoer word by die Universiteit van Johannesburg om vishistologie in toksisiteitstudies in Suid-Afrika te bevorder. Die resultate verskaf die volgende: **(1)** kennis van die histologie van twee varswater indikator visspesies wat nog nie voorheen in diepte beskryf is nie; **(2)** dra by tot 'n beperkte hoeveelheid inligting beskikbaar van die normale histologie van Suider-Afrikaanse visspesies; **(3)** verskaf verwysingsmateriaal vir teikenorgane in vishistopatologie met die toepassing van 'n standaard assesseringsmetode aan die hand van spesifieke kriteria om meer akkurate resultate te verseker; **(4)** dra by tot die uiteindelijke skepping van 'n omvattende varswater vishistologie atlas vir Suider-Afrikaanse spesies; **(5)** verskaf verwysingsmateriaal vir somatiese indekse, spesifieke bloed parameters en kondisie faktor; **(6)** en verskaf 'n werksdokument om gebruik en aangevul te word deur toekomstige histologiese studies, vir basiese histologie opleiding in die Department Dierkunde van die Universiteit van Johannesburg.