Development of a Quality Index Method (QIM) scheme and its implementation in a shelf-life study of kingklip (*Genypterus capensis*)

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Abstract

In this study Quality Index Method (QIM) schemes for raw headed and gutted (H&G) fish and skinless fillets as well as cooked fillets of kingklip (*Genypterus capensis*) were developed in a shelf life study. The QIM schemes for raw H&G and skinless fillets are based on six and seven quality attributes respectively, with a scoring system from 1 to 6, where a score of six is best quality (perfect). For the case of cooked fillets the scheme is based on six attributes, with a scoring system from 1 to 5, where a score of five is best quality. Sensory analysis of cooked fillets was carried out parallel to sensory analysis of raw H&G and skinless fillets as well as microbial count study until 18 days of storage. These were used to decide the maximum storage time in ice and to observe how the different quality attributes of cooked fish, especially odour and flavour, deteriorated with storage time in ice, as they are the best symptoms of fish spoilage. However, the microbial count study alone was continued until 22 days in ice in order to follow the bacterial spoilage pattern with storage time.

In the study, a total of 94 fish were used: 54 fish (108 fillets) for the development of the QIM scheme and 40 fish (half of which were filleted) with a mean weight and standard deviation of 570.22g (± 114.41) were used in the course of an independent storage trial in a shelf-life study. The fish are gutted and headed on board the fishing vessels and hence the study was conducted on H&G fish and fillets. They were stored in a chiller at 0-2 °C, packed with ice in aerobic conditions for up to 18 days and sampled by taking four fish (two H&G and four fillets) every day during the first three days and thereafter, every second or third day until 18 days of storage.

The shelf-life study of kingklip resulted in a negative linear relationship between QIM scores for raw H&G and skinless fillets as well as cooked fillets ($R^2 = 0.981$, $R^2 = 0.973$ and $R^2 = 0.873$ with a slope of -0.667, -0.839 and -1.242, respectively) with storage time. Based on sensory evaluation of fresh H&G and skinless fillets as well as cooked fillets, the shelf-life of kingklip was determined as 18 days in ice, but taking the palatability of the cooked fish into account the shelf life was found to be 14 days. From statistical analysis with one-way ANOVA, the mean QI scores for raw H&G and skinless fillets as well as cooked fillets were found to be significantly different with storage time ($p < 0.05$,

n = 57). Assessors participating in the sensory evaluation with the QIM scheme performed differently and this was further tested using two-factor design with interaction in ANOVA and it was found that there was a significant difference among assessors ($p < 0.05$, n = 224 (for fresh H&G), n = 228 (for fresh skinless fillets), n = 342 (for cooked fillets)). This implies that the freshness assessment with the QIM scheme should be based upon the assessment of more than one assessor and all should be well trained in order to reduce the variation.

In order to attempt to understand the rate of spoilage in kingklip during storage in ice, bacterial counts were carried out. Total viable count (TVC), selective counts of H_2S-producing bacteria (*Clostridium perfringens*), Coliforms and *Listeria monocytogenes* were done on skin and flesh samples (from the loin and tail parts) of H&G and flesh samples of skinless fillets. The TVC count increased with storage time in ice and was ca. 10^6 cfu/g on skin of H&G and flesh of fillet and ca. 10^5 cfu/g in flesh samples (from the loin and tail parts) of H&G after 22 days of storage in ice. The TVC count on skin of H&G was low compared to similar studies made, where the TVC count on skin of H&G was found to be ca. 10^8 cfu/cm^2. The reason for the low value of TVC on skin of H&G and flesh of skinless fillets could probably be because kingklip may develop inhibitors against bacterial growth. Analysis of *C. perfringens*, Coliforms and *L. monocytogenes* were all found to be negative, indicating that the process does not appear to add extra species of bacteria to the fish.

1. Introduction

1.1. Sensory evaluation of raw fish freshness

Purchase and management of the raw material during storage are fundamental parts of production planning in the seafood industry (Hansen 1991, as cited in Nielsen *et al.* 1992; Jonsdottir *et al.* 1999). Reasonably precise information on the storage history of the raw material is a necessary adjunct to the processing of high quality products (Lougovois *et al.* 2003). As seafood spoils, it goes through a sequence of changes that are detectable by the human senses. Sensory evaluation is defined as "the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing" (Stone and Sidel 1985; Jonsdottir 1992; Nielsen 1995a, 1997; Olafsdottir *et al.* 1997). This definition was prepared by the Sensory Evaluation Division of the Institute of Food Technologists (Anon 1975) and also quoted by Stone and Sidel (1985).

Storage life (shelf-life) or storage time refers to the degree of spoilage beyond which most customers would reject the fish; and the storage life for fish to remain of good eating quality is about half the total storage life (Howgate 1982). The most dramatic change in fish muscle is the onset of rigor mortis. Immediately after death, the muscle is totally relaxed and the limp elastic texture usually persists for some hours, whereafter the muscle will contract. When it becomes hard and stiff, the whole body becomes inflexible and the fish is said to be in rigor mortis or simply in rigor. This condition usually lasts for a day or more and then rigor resolves, the stiffening lessens and the fish is again limp, but no longer as elastic as before rigor (Hobbs 1982; Nielsen 1995b). The body of a fresh fish after resolution of rigor is firm and elastic to the touch. The rate in onset and resolution of rigor varies from species to species and is affected by temperature, handling, size and physical condition of the fish (Nielsen 1995b). On storage, fish muscle becomes softer and finger indentations are retained (Howgate 1982).

The colours of a fresh fish are bright and the skin has a clear shiny appearance, but the colours fade and become duller on storage until they are ultimately hidden by bacterial slime. The fillet of a fresh fish has a glossy appearance and the flesh is somewhat translucent, but fillet cut from a fish stored for a few days in ice is dull and waxy looking

(Howgate 1982). The texture of a raw fish softens during chilled storage because proteolytic enzymes break down the muscle structure (Andersen 1995, as cited in Sveinsdottir *et al.* 2002).

Freshness is a property of fish considered to be one of the most important factors in determining the overall quality of raw fish (Jonsdottir 1992). Indeed it is the most important single criterion of quality for the majority of fish products. This, bearing in mind the high perishability of fish, makes it essential that anyone concerned with the quality of fish and fish products must be able to determine freshness (Howgate 1982). The most common methods used to evaluate freshness of fish are sensory evaluation methods (Sveinsdottir *et al.* 2002; Barbosa and Vaz-Pires 2003). Sensory evaluation methods are commonly used in the fish sector and fish inspection services (Hootman 1992; Luten and Martinsdottir 1997; Martinsdottir 1997; Hyldig and Nielsen 2001) and they are the most important methods for freshness evaluation in the fish research (Martinsdottir 1997). Loss of freshness of seafood is a consequence of post-mortem biochemical, physicochemical and microbiological processes characteristics of each species, of handling on board and on land, and of technological processing. These changes are appreciable in sensory terms (Huidobro *et al.* 2000).

Sensory methods are the oldest and still the most satisfactory way of grading and assessing the freshness of fish and fish products (Howgate 1982; Branch and Vail 1985; Howgate *et al.* 1992). They can be applied to all species of fish, need no laboratory facilities, are quick, non-destructive and are closely related to the criteria the consumer uses in evaluating acceptability (Howgate 1982). Sensory methods relying on trained assessors, i.e. objective sensory methods, are required for use in quality control for evaluation of freshness and for determination of remaining shelf life of seafoods (Delgaard 2000). Sensory methods performed in a proper way are a rapid and accurate tool providing unique information about food. They offer immediate measurement of perceived attributes (Hyldig and Nielsen 2001).

The purpose of the sensory evaluation of the raw fish is to describe (literally) all-detectable aspects of changes on the fresh fish during cold storage in ice. This involves a very detailed description of all possible changes of sensory parameters: appearance, texture and odour. In consequence every deviation for a specific parameter - e.g. the firmness of the flesh - has to be thoroughly described in common words for each new evaluation during the storage trials (Nielsen *et al.* 1992; Hyldig and Nielsen 1997; Jonsdottir *et al.* 1999).

1.1.1. History of sensory evaluation methods

Consumer acceptance of food products is determined by sensory quality. It is therefore extremely useful to have methods for describing the sensory properties as a means of ascertaining their initial sensory characteristics and any changes undergone by the product in the course of storage. For decades now, sensory analysis has been one of the key criteria in defining the quality of most fishery products (Huidobro *et al.* 2000) and it has for years played a natural part of the fishery chain (Hyldig and Nielsen 2001).

A large number of schemes have been developed for sensory analysis of raw fish during the last 50 years (Nielsen 1995a; Hyldig and Nielsen 1997) and use of effective sensory methods is essential for successful production planning in quality control (Jonsdottir *et al.* 1999). The first modern and detailed sensory method for the evaluation of raw fish was developed at the Torry Research Station (Shewan *et al.* 1953). The basic idea was that each quality parameter was independent of other quality parameters. Then the quality attributes were given scores (Nielsen 1995a). Characteristic sensory changes occur in appearance, odour, taste and texture of fish when they deteriorate (Shewan *et al.* 1953). Today in Europe, the most commonly used methods for quality assessment of raw fish in the inspection service and in the fish industry are the EU Freshness Grading or the EC-scheme (Jonsdottir 1992; Larsen *et al.* 1992; Nielsen 1995a; Anon 1996, as cited in Olafsdottir *et al.* 1997; Barbosa and Vaz-Pires 2003) and the Quality Index Method (QIM) (Bremner 1985), which is a recent scheme now coming out of its research phase into commercial practice (Luten and Martinsdottir 1997; Nielsen 1997; Olafsdottir *et al.* 1997; Huidobro *et al.* 2000; Martinsdottir *et al.* 2001, as cited in Barbosa and Vaz-Pires 2003; Sveinsdottir *et al.* 2002; Barbosa and Vaz-Pires 2003).

The EU scheme was introduced for the first time in the Council Regulation no. 103/76 (Jonsdottir 1992; Larsen *et al.* 1992; Nielsen 1995a) and updated by decision no. 2406/96, which includes some of the improvements published by Howgate *et al.* (1992). There are three levels/ grades in the EU scheme, E (Extra, the highest quality), A (good quality), B (satisfactory quality). Below level B (sometimes called Unfit or C) fish is not acceptable for human consumption, thus it is discarded or rejected (Howgate 1982; Jonsdottir 1992; Larsen *et al.* 1992; Nielsen *et al.* 1992, 1995a; Hyldig and Nielsen 1997, 2001; Barbosa and Vaz-Pires 2003).

The EU scheme is not designed to be used in quality assurance in the processing industry, where the users attach numbers to the grades and carry out arithmetic on these numbers (Hyldig and Nielsen 2001). It is commonly accepted for sensory assessment in the EU countries. However, its validity has been questioned since there is still some discrepancy as it does not take clearly into account differences between species and it only uses general parameters of appearance for describing the changes in iced fish (Jonsdottir 1992; Nielsen *et al.* 1992; Nielsen 1995a, 1997; Hyldig and Nielsen 1997; Luten and Martinsdottir 1997; Olafsdottir *et al.* 1997; Delgaard 2000; Barbosa and Vaz-Pires 2003) resulting in a classification within four groups (E, A, B and C) and giving a considerable freedom of interpretation to the individual assessor (Jonsdottir 1992; Nielsen *et al.* 1992).

According to Delgaard (2000), the EU sensory scheme has disadvantages because information on remaining shelf life cannot be obtained directly from the freshness grades and, because the schemes are too complicated, they may not be followed in practice. Therefore, as an alternative to EU scheme, a new improved seafood freshness quality grading system, the Quality Index Method (QIM) has been suggested and developed for evaluation of fresh fish in production management, in seafood inspection and other parts of the fishery chain (Hyldig and Nielsen 2001; Barbosa and Vaz-Pires 2003).

1.1.2. The Quality Index Method (QIM)

Because of the increased trade between countries, purchases are often performed on unseen lots, and there is a need for a freshness grading system such as the Quality Index Method (QIM). This method is a seafood freshness quality grading system, which is used to assess fish freshness in a rapid and reliable way (Sveinsdottir *et al.* 2002). QIM for whole fresh fish is based upon a scheme originally developed by the Tasmanian Food Research Unit in the mid 80's (Bremner 1985; Bremner *et al.* 1987, as cited in Warm *et al.* 1998; Branch and Vail 1985), but needs to be developed separately for each fish species (Hyldig and Nielsen 1997; Sveinsdottir *et al.* 2003). It was further developed in the Nordic countries (Larsen *et al.* 1991, 1992; Jonsdottir 1992), in the EU (Nielsen 1993, as cited in Warm *et al.* 1998) and at the Danish Institute for Fisheries Research (Nielsen *et al.* 1994; Warm *et al.* 1998). It is based on characteristic changes that occur in raw fish (Branch and Vail 1985; Bremner 1985; Olafsdottir *et al.* 1997; Sveinsdottir *et al.* 2002).

QIM is a rapid grading method, which establishes more exact data reflecting the different quality levels of fish in a simple and well-documented way for production management and fish inspection (Hyldig and Nielsen 1997, 2001; Jonsdottir *et al.* 1999) and has been suggested as a new standard method (Hyldig and Nielsen 1997). It is based upon objective evaluation of significant key sensory attributes for whole raw fish using many but weighted parameters in the deterioration of seafood (Larsen *et al.* 1992; Nielsen *et al.* 1992, 1994; Hyldig and Nielsen 2001; Sveinsdottir *et al.* 2003). It is a new tool, by which sensory assessment is performed in a systematic and reliable way and used as a truly objective analysis (Hyldig and Nielsen 2001). The technique is based on selecting a number of quality attributes characteristic for a particular species and allocating scores to each attribute depending on the state of freshness or quality of the selected food item. The scores are assigned in whole numbers ranging from 0 (for fresh) to 3 or 4 demerit (index) points pending advancement of decay (Branch and Vail 1985; Bremner 1985; Bremner *et al.* 1987, as cited in Warm *et al.* 1998; Jonsdottir 1992; Hyldig and Nielsen 1997, 2001; Larsen *et al.* 1992; Nielsen *et al.* 1992, 1994; Sveinsdottir *et al.* 2003), i.e. a QIM scheme gives scores of zero for very fresh fish and an increasingly larger total as the fish deteriorate (Hyldig and Nielsen 1997, 2001; Jonsdottir *et al.* 1999). The selection of parameters in QIM schemes is determined as a combination of the best descriptors for the spoiling fish (Hyldig and Nielsen 2001). Score points are assigned for changes in outer appearance of eyes, skin, gills and changes that occur in odour and texture (Hyldig and Nielsen 1997; Jonsdottir *et al.* 1999; Huidobro *et al.* 2000; Sveinsdottir *et al.* 2002, 2003).

By assessing many parameters in a QIM scheme, no single parameter can unduly imbalance the score, i.e. no undue emphasis is placed on a single feature. A sample cannot be rejected on the basis of a single criterion and minor differences in judgments in any one criterion being assessed do not unduly influence the total score (Branch and Vail 1985; Bremner 1985; Jonsdottir 1992; Botta 1995; Hyldig and Nielsen 1997; Luten and

Martinsdottir 1997; Martinsdottir 1997; Nielsen *et al.* 1992, 1994; Nielsen 1997; Warm *et al.* 1998; Huidobro *et al.* 2000).

QIM uses a practical rating system, in which the fish is inspected and the characteristics listed on the scheme are assessed and the fitting demerit point scores are recorded (Luten and Martinsdottir 1997). The scores for all the characteristics are then added to give an overall sensory score, the so-called Quality Index (QI) (Jonsdottir 1992; Nielsen *et al.* 1994; Hyldig and Nielsen 1997, 2001; Olafsdottir *et al.* 1997; Jonsdottir *et al.* 1999; Delgaard 2000; Huidobro *et al.* 2000; Sveinsdottir *et al.* 2003). The QI score increases linearly with keeping time in ice. Therefore the total demerit score or QI can be used to predict the remaining shelf life (storage life) of fresh fish stored at 0°C (Jonsdottir 1992; Larsen *et al.* 1992; Hyldig and Nielsen 1997, 2001; Luten and Martinsdottir 1997; Olafsdottir *et al.* 1997; Delgaard 2000; Sveinsdottir *et al.* 2002).

For validation of a QIM scheme, there must be more than four assessors and a new batch of fish from the same lot should be used daily for every assessment during the storage trial period (Jonsdottir *et al.* 1999). The aim of a QIM scheme is to achieve a linear correlation between the sensory quality expressed as a sum of demerit scores or QI and storage life on ice (Branch and Vail 1985; Jonsdottir 1992; Larsen *et al.* 1992; Nielsen *et al.* 1992, 1994; Hyldig and Nielsen 1997, 2001; Sveinsdottir *et al.* 2002). In addition, the QIM is usable or valid in the first 75% of the storage period, whereas other instrumental methods (physical, chemical and microbiological) are inaccurate and unreliable in the first 40% of the storage period (Larsen *et al.* 1992; Nielsen *et al.* 1992).

Most of the sensory evaluation in the fish industry is either done on whole intact fish or on raw fillets. The greatest advantage of the QIM is the carefully planned scoring system with valid parameters making the results of the sensory evaluation credible (Luten and Martinsdottir 1997). QIM is primarily used in the evaluation of whole and gutted fish and also according to Olafsdottir *et al.* (1997) the grading of raw fillets occurs in the fish industry, but it is more common to cook fillets before carrying out sensory evaluation. However, according to Delgaard (2000), it is difficult to use the QIM scheme with fish fillets and schemes for lightly preserved seafoods are not yet available.

In recent years, QIM schemes have been developed for a number of different species, taking into account the intactness of the fish (whole, gutted, fillets) and the technological treatment used (Huidobro *et al.* 2000). QIM schemes have been developed for the following fish species: round fish, spotty trevalla (*Seriolella punctata*) (Branch and Vail 1985); Saithe (*Pollachius virens*), raw intact plaice (*Pleuronectes stellatus*) and anchovy (Nielsen *et al.* 1992); fresh herring (*Coupe harengus*) and cod (*Gadus moruha*) (Jonsdottir 1992; Larsen *et al.* 1992), red fish (*Sebastes mentella/marinus*) (Martinsdottir and Arnason 1992, as cited in Sveinsdottir *et al.* 2003), Atlantic mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*) and European sardine (*Sardina pilchardus*) (Nielsen *et al.* 1992; Andrade *et al.* 1997), brill (*Rhombus laevis*), dab (*Limanda limanda*), haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), sole (*Solea vulgaris*), turbot (*Scophtalmus maximus*) and shrimp (*Pandalus borealis*) (Luten 2000), gilthead seabream (*Sparus aurata*) (Huidobro *et al.* 2000); trout (Hyldig and Nielsen 2001); farmed salmon (*Salmo salar*) (Sveinsdottir *et al.* 2003) and for common octopus (*Octopus vulgaris*) (Barbosa and Vaz-Pires 2003).

1.1.3. Sensory assessment of cooked fish

Sensory assessment can be extended to the cooked fish. When a fish product is in the form of fillets or headless fish, examination of a cooked sample is required for a reliable assessment of freshness (Howgate 1982). A clear definition of the time for rejection is a step needed for a QIM scheme development. The rejection for fish species has been defined using sensory assessment of cooked flesh i.e. the maximum storage time of fish can be determined by sensory evaluation of cooked samples (Sveinsdottir *et al.* 2002; Barbosa and Vaz-Pires 2003).

The sensory analysis of cooked fish is based on judgement of taste/flavour, texture and odour from a trained panel (Jonsdottir 1992; Nielsen *et al.* 1992; Jonsdottir *et al.* 1999). A descriptive 10-point scale developed at the Torry Research Station is often used for this purpose. This scale is often referred to as the Torry scale (Shewan *et al.* 1953). It has been developed for lean, medium fat and fat fish species and scores are given from 10 (very fresh in taste and odour) to 3 (spoiled). The Torry scheme is the first detailed scheme developed for freshness evaluation and it is the most commonly used scale for the freshness evaluation of cooked fish both in the fish industry, where sensory evaluation of fillets is needed, and in research laboratories throughout Europe (Olafsdottir *et al.* 1997). It provides limited information about how the individual characteristics of the cooked fish change through the storage time, but by using Quantitative Descriptive Analysis (QDA) methods, much more detailed information can be gained (Sveinsdottir *et al.* 2002).

The Quantitative Descriptive Analysis (QDA) (Stone and Sidel 1985) is a sensory method, which may be used for the determination of maximum shelf life in addition to a detailed description of the sensory profile for a product. With QDA, all detectable aspects of a product are described and listed by a trained panel. The list is then used to evaluate the product, and the panellists quantify the sensory aspects of the product using an unstructured scale (Sveinsdottir *et al.* 2002).

Cooked fish flavour is an excellent indicator of freshness quality and may provide precise information on the time of storage following harvest. However, especially trained expert assessors are required for objective sensory evaluation (Botta 1995) and this can be both expensive and inconvenient (Connell 1995).

1.2. Non-sensory methods of freshness assessment

Non-sensory methods for evaluating fish quality include microbial counts (Total Viable Count (TVC) and hydrogen-sulphide producing bacteria (HPB)); physical e.g. pH, "Fish Tester" value, "Torrymeter" readings, and "RT-Freshness Grader" readings; and chemical e.g. TMA (trimethylamine) and K-value (Jonsdottir 1992). In this study only the microbial count was conducted. The chemical methods for evaluating fish quality were not done since from I&J's perspective, fish harvested from South African waters have very low levels of TMA and TVBN (Total Volatile Bases of Nitrogen) (Per. Comm. Graz 2003).

1.2.1. Microbial counts

About one-third of the world's food production is lost annually as a result of microbial spoilage (Lund *et al.* 2000, as cited in Delgaard 2000). Microorganisms are the most important agents of deterioration in raw, wet fish, since they give rise to the particularly undesirable flavours associated with spoilage (Connell 1990). In fact, microbial activity is responsible for spoilage of most fresh and several lightly preserved seafoods (Delgaard 2000) and is the main factor limiting the shelf life of fresh fish (Howgate 1982; Olafsdottir *et al.* 1997). The remaining shelf life is used as a definition of fish freshness (Olafsdottir *et al.* 1997). Possibly for this reason, the total viable counts (TVC) or aerobic plate counts (APC) have been used in mandatory seafood standards in some European Countries, in Japan and the USA. Furthermore, TVCs are used extensively in microbiological specifications and as an acceptability index in standards and guidelines as part of purchase agreements (Olafsdottir *et al.* 1997; Delgaard 2000). However, only a small fraction of the microorganisms present on newly processed seafood is actually of importance for product spoilage (Delgaard 2000).

Spoilage begins as soon as fish die and the spoilage mechanism is very complex, but the main factors can be attributed to microbial action or enzymatic/chemical activity (Nielsen *et al.* 1992). During storage of seafood at particular conditions of temperature, atmosphere, % salt, water activity (a_w) and preservation, specific spoilage organisms (SSO) grow faster than the remaining seafood microflora, and eventually produce the metabolites responsible for off-flavours and sensory product rejection. Consequently, the numbers of SSOs and the concentration of their metabolites can be used as objective quality indices for shelf life determination in seafoods (Delgaard 2000).

The end of shelf life is the result of unpleasant sensory characteristics not caused by chemical changes, but mostly due to bacterial growth activities (Howgate 1982). Newly caught fish contain a diverse microflora and the amount of bacteria can vary greatly, normally ranging from10^2 to 10^7 cfu (colony forming units) /cm^2 (Liston 1980, as cited in Sveinsdottir *et al.* 2002). During ice storage the bacteria will grow with a doubling time of approximately one day and after 2-3 weeks, reach numbers of 10^8-10^9 cfu/g flesh or cm^2 skin (Gram 1995a). As the spoilage of iced fish is caused by specific spoilage bacteria (Liston 1980, as cited in Sveinsdottir *et al.* 2002), several methods have been developed for specific determination of such bacteria in fresh fish. It has been reported that the expected shelf life of chilled cod fish can be predicted by the number of specific spoilage bacteria (Ravn-Jorgensen *et al.* 1988) and the development of rapid methods for detection of these bacteria is therefore of particular interest (Gram 1992).

The most important seafood spoilage bacteria in marine fish are characterized by their ability to produce H_2S (hydrogen-sulphide) and reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), which on its own is always recognized as being ammoniacal, and has been used for their specific determination (Gram *et al.* 1987; Connell 1990; Olafsdottir *et al.* 1997; Sveinsdottir *et al.* 2002). Capell *et al.* (1997) found counts of H_2S-producing bacteria (HPB) to be more closely associated with the rejection of several fish species, irrespective of the temperature and atmosphere, than the total viable count. The bacterium *Shewanella putrefaciens*, which produces hydrogen sulphide, was determined as the specific spoilage organism (SSO) on some chilled fresh fish (Olafsdottir *et al.* 1997).

Counts of H_2S-producing bacteria, though they constitute a small proportion of the total aerobic bacteria, provide a useful indicator of quality deterioration and could be used to determine the time to rejection, while total counts at 20°C are only poor measures of freshness quality (Lougovois *et al.* 2003). According to Olafsdottir *et al.* (1997), TVC's of 10^2-10^6 cfu/g are common on whole fish, but at the point of sensory rejection, the TVC of fish products are typically 10^7-10^8 cfu/g. Nevertheless, standards, guidelines and specifications often use much lower TVC as indices of acceptability, e.g. a TVC of 10^6 cfu/g is used as a index of acceptability by I&J for its production aiming for local and export markets, which is in line with the South African Bureau of Standards (SABS) (Per. Comm. Graz 2003).

The aim of microbiological examinations of seafood products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality of the seafood including temperature abuse and hygiene during handling and processing (Gram 1995b). The determination of bacterial numbers or bacteriological examination in seafood is widely used as an indicator of hygiene (Olafsdottir *et al.* 1997; Howgate 1982).

Fish, like other living animals, carry a number of bacteria, called the resident flora, on the outer surfaces and in the intestines (gut). The normal population or flora consists of several groups or genera of microorganisms (Connell 1990). In a normal healthy fish, there are a variety of types of bacteria present on the skin, gills and in the intestines that cause no harm to the fish. The flesh is sterile in a live healthy fish since the bacteria are kept from invading the sterile fish by the fish's normal defences or immune system (Gram 1995a). Once the fish is caught and dies, the immune system collapses and bacteria, or the enzymes they secrete, are able to penetrate and invade the flesh by moving between the muscle fibres, where they degrade tissue components to produce unpleasant odours and flavours associated with spoilage (Hobbs 1982; Howgate 1982; Connell 1990; Gram 1995a). The numbers of microorganisms in the fish flesh grow slowly initially but then increase rapidly (Connell 1990). The changes brought about by catching, handling and processing will determine which types of bacteria continue to grow. In addition, handling and processing will remove some bacteria, kill others and add new species to the fish (Hobbs 1982).

Bacteria causing spoilage in fish are psychrophilic; a group that grows well at relatively low temperatures (-5°C) and grows best at 15-20°C, but not above 30-40°C. These bacteria grow well in fish, whose pH is usually between 6.4 and 6.8 (Hobbs 1982). Any odour and flavour changes in iced fish during the first 2-4 days after death are autolytic and generally do not involve bacteria. After this stage, the bacterial population recovers from the shock of the change in the environment and those able to grow in the new situation begin to do so. Most of the undesirable odour and flavour changes associated with spoiling fish result from bacterial action, some of which may arise by previous autolytic reactions. Not all the bacteria able to grow produce changes; spoilage odours and flavours are produced by members of the genus *Pseudomonas* (Hobbs 1982). A closely related contemporaneous sequence of changes occurs in the odour of the external surfaces and gills or organs. These odours are more intense than those in the flesh and can be used as excellent indices of degree of spoilage (Connell 1990).

The broad pattern of changes in the bacterial flora developing on spoiling fish in ice is the same irrespective of species or where the fish were caught, yet organoleptically there are large differences in the spoilage pattern of different fish; these differences are probably a result of differences in the chemical composition of fish (Hobbs 1982). The bacterial spoilage potential of any fishery product will depend on the initial flora of the raw materials, the conditions prevailing during processing and the subsequent storage conditions (Hobbs 1982).

1.3. Kingklip

The genus *Genypterus* (to which kingklip, *Genypterus capensis,* belong) is found only in southern hemisphere temperate waters and is more commonly known worldwide as ling (Japp 1990). Kingklip, *Genypterus capensis* are deep-water benthic fish, endemic to southern Africa. Unlike hake and sole, which are cosmopolitan, they are only found in southern hemisphere (Payne and Badenhorst 1989). Their distribution extends from North of Walvis Bay on the coast of Namibia to east of Port Elizabeth on the South African south coast (Fig. 1) (Smith and Heemstra 1986; Punt and Japp 1994). They are long-lived, slow-growing and have been aged to 24 years (Japp 1990).

Assessment of the kingklip resource is complicated by lack of agreement among fisheries scientists as to how many stocks exist off southern Africa. There is more than one stock of kingklip around southern Africa. That caught from Luderitz northwards has a leaner, blunter appearance and the colour is more brown than the bright pink of specimens caught from just north of the Orange River to beyond Port Elizabeth (Payne and Badenhorst 1989). Japp (1989, as cited in Punt and Japp 1994) recommended that, until the stock-identity question has been resolved satisfactorily, the kingklip resource harvested off South Africa be managed as two units, i.e. West and South stocks.

Kingklip are the second most valuable stock of ground fish species, but they are of higher value in terms of unit fish price per kg than hake. Successful attempts have been made to establish a directed fishery for the species, but it still constituted only a minor portion of the total ground fish catch because of the dominance of hake. For many years, they were taken almost entirely as by-catch in the hake trawl fishery (Payne and Badenhorst 1989). They have always constituted a small but important by-catch of the directed trawl fishery for the Cape hakes *Merluccius paradoxus* and *M. capensis* off South Africa (Botha 1970, as cited in Punt and Japp 1994). However, from 1983 to 1990 they were also the target of a directed longline fishery (Badenhorst 1988).

Kingkilp used to be taken by a directed longline fishery as well as by trawl. Although they are occasionally caught in shallow water, most catches by trawl and longline are made in deeper water, from 250-400 m (Payne and Badenhorst 1989).

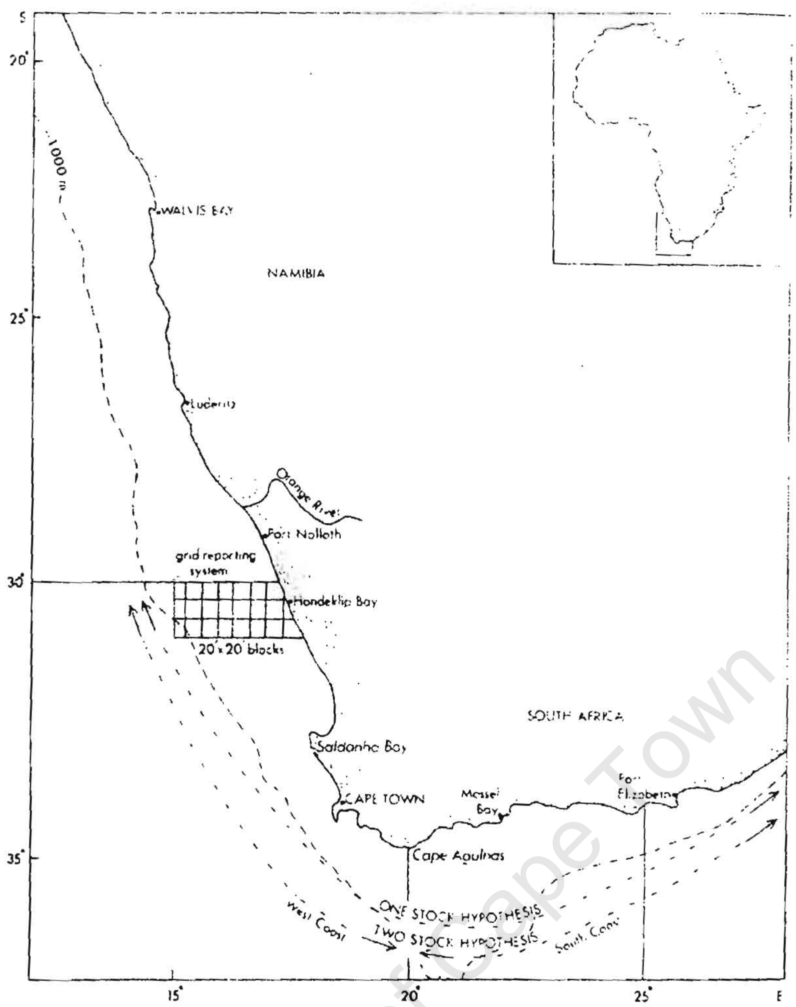


Figure 1. Map of southern Africa showing the place names mentioned in the text (Source: Punt and Japp 1994).

1.3.1. Life History

Kingklip probably prefer life in rocky areas and they have planktonic larvae, but the young fish soon sink to the bottom. Small fish are occasionally caught by trawlers, especially close in-shore and adults are taken more often in deeper water, so it may be assumed, as with most fish species, that there is a migration to deeper water as the fish grows (Payne and Badenhorst 1989).

The age at which kingklip become sexually mature is variable in different parts of their range, but it is generally at 4-6 years (45-75 cm long). Catches of fish more than 1 m long are common. The actual spawning season is believed to be protracted on the South Coast, but somewhat shorter on the West Coast. The peak tends to be in spring on the South Coast, but there is also quite heavy activity in late winter (Payne and Badenhorst 1989).

The size of the fishing grounds and trends in catches seem to point to a small stock incapable of producing much more annually than the average catch over the last few decades (Payne and Badenhorst 1989).

1.3.2. History of the fishery

Over the period 1932-1944, kingklip in hake-directed trawls averaged 615 tons per annum. The bulk of this catch (80%) was taken off the West Coast (Scott 1950, as cited in Punt and Japp 1994; Chalmers 1976). The average catch increased to 1,857 tons for the years 1945-1965, with a slightly lower proportion taken off the West Coast (70%). Trawled landings of kingklip between 1966 and 1983 continued to increase (average of 3,867 tons) peaking at 5,800 tons in 1973 (Fig. 2) (Badenhorst 1988).

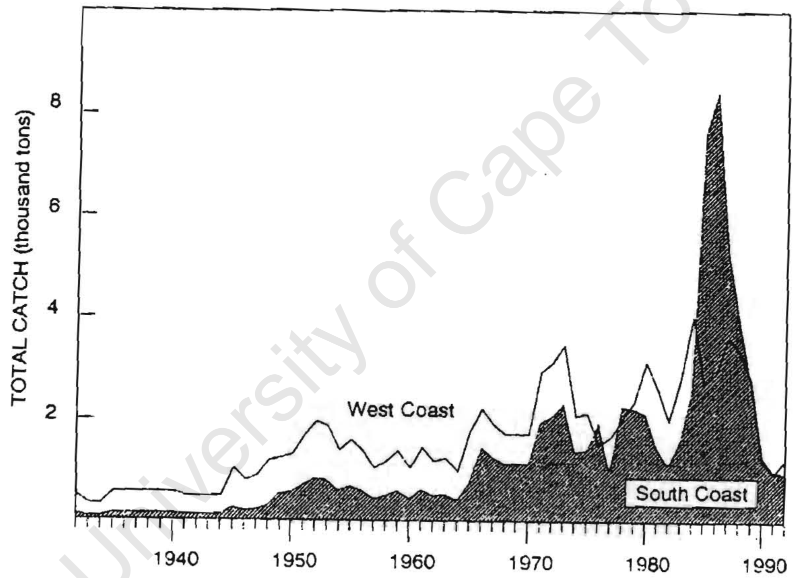


Figure 2. Catches of kingklip off South Africa from 1932 to 1992 on the West and South coasts (Source: Punt and Japp 1994).

The longline fishery was initiated off South Africa in 1983 to selectively harvest the hake resource (Badenhorst 1988; Booth and Hecht 2000). Soon after its inception, the fishery switched to kingklip (Booth and Hecht 2000). Catches were high and resulted in a sharp increase in total landings of the species. Longline catches of kingklip peaked in 1986, most of the catch being taken on the South Coast (80%). Longline and trawled catches declined sharply thereafter (Japp 1989) due to the high directed fishing pressure on the spawner stock, which in retrospect, was already overfished (Punt and Japp 1994).

In terms of catch rate in the longline fishery (kg·1000 hooks^{-1}, after Badenhorst 1988), the data show a distinctly seasonal pattern. The principal reasons for this is that kingklip form spawning aggregations on the South Coast from late winter (August) to well into spring (Scott 1950, as cited in Punt and Japp 1994; Japp 1989) and were targeted by the longline fleet at this time. In South African waters, spawning aggregations of kingklip apparently only occur on the South Coast, particularly on the eastern side of the Agulhas Bank. A second factor that may enhance the catchability of kingklip to both the trawl and longline gear in spring is the slackening of the strongly-flowing Agulhas Current at the same time as the aggregations (Scott 1950, as cited in Punt and Japp 1994), thereby better facilitating longlining and trawling than at other times of the year. Both the longline and trawl fisheries take advantage of the aggregations and target intensively on the species from midwinter to spring (Japp 1989, as cited in Punt and Japp 1994).

As a result of the assessments done, as well as growing discontent among trawl fishermen at the sharp reduction in kingklip by-catch (which was attributed to the systematic removal of the kingklip spawner biomass by the longline fleet), the kingklip-directed longline fishery was closed in 1991 (Punt and Japp 1994; Booth and Hecht 2000).

Despite the commercial importance of the species, no studies have investigated the changes occurring in kingklip through typical handling, distribution and storage conditions up to now.

1.3.3. Kingklip harvesting, processing and marketing

The companies which are the main exporters of kingklip in South Africa are Irvin and Johnson (I&J), Seaharvest, Blue Continent Product (BCP) and Kaytrad. Generally, there is no quota limit for harvesting kingklip as it is only caught as a by-catch in the hake trawl and longline fishery. Kingklip are processed by I&J chilled in the form of skinless fillets and portions, and frozen in the form of H&G and portions. Out of the total production of kingklip by I&J, 70% is exported to European Union (EU) member countries and the Far East and 30% (mainly H&G and fillet portions) is sold locally in South African markets (Per. Comm. Graz 2003).

1.4. I&J

I&J is the leading frozen food marketing and distribution company in South Africa. The major source of supply of products marketed by the seafoods division is caught by I&J's own fleet of trawlers, which is one of the largest fishing fleets in the southern hemisphere. Headquartered in Cape Town, I&J has offices, factories, fishing fleets, branches and subsidiaries throughout the country, as well as international representation in Australia, the Pacific Rim, the United States, Europe and Africa. Fleets are based in Cape Town, Mossel Bay, Durban and Port Elizabeth (squid). Fish processing and freezing is carried out in Cape Town, Mossel Bay (hake and linefish), Walvis Bay in Namibia (hake and pilchard) and Hermanus factories, as well as at sea.

1.4.1. Seafood trawling

I&J operate one of the largest trawling fleets in the Southern Hemisphere, fishing in an area of some 122,400 square miles in the Indian and Atlantic Oceans. It holds a substantial portion of South Africa's 180,000 ton hake quota. The company's fleet of 38 vessels includes factory/freezer, wet fish and Crustacea trawlers and the company trawls manly for Hake (*Merluccius capensis* and *M. paradoxus*) in South African and Namibian waters and also fishes kingklip (*Genyperus capensis*) and various other species of local line fish. All I&J's vessels and factories are HACCP (Hazard Analysis Critical Control Point) compliant so as to fulfil the requirements for exports anywhere around the world.

The company's seafood division has a total of 3,800 employees in the whole of South Africa and some 1,500 of these employees working over two shifts are based at the company's major fish processing factory in Woodstock, Cape Town (where this study was conducted). This processing company produces a diverse range of approximately 100 different frozen fish products using hake as a base raw material. The range includes natural cuts such as hake fillets, cutlets, steaks and loins. The factory's production capacity is approximately 120 m tons of hake and 4-6 m tons of kingklip per day. The main products made using kingklip as a raw material are kingklip fillets, portions that could be either chilled or frozen (Per. Comm. Graz 2003).

1.4.2. I&J's use of Quality Index (QI) for hake

I&J has already developed a Quality Index (QI) scheme for hake, *Merluccius capensis* H&G and skinless fillets. The scheme is similar to the QIM scheme discussed above, but it does not include organoleptic assessments and the scales/demerit score points used for grading/evaluating the hake H&G and fillets are 1-6 rather than the 0-3 or 0-4 scores, which are normally used in the case of QIM scheme for other species in other countries.

The main aim for developing the QI scheme for hake was to evaluate the incoming raw material (hake being the main raw material) by sampling 10% of each vessel's catch and for use in production planning and quality management. Furthermore, it is used in allocating the raw material into the five different factories within the seafood processing plant in order to produce first grade fish product based on the developed grading system and to monitor the performance of the fishing vessels i.e. to give a feedback to the trawlers (trawling division) about the quality of their catch and to make recommendations in order to improve the fish handling on board.

The quality parameters/defects that were included in the QI scheme for evaluating hake fillets were discolouration, blood on the fat line, blood clots, gaping, parasite occurrence and softness/texture of the fillet. While for the case of evaluating hake H&G, the quality defects that were included in the QI scheme were skin damage, colour of the neck end (brown neck), incorrect deheading, rugged neck and odour of the fish. The main reasons for choosing the above mentioned parameters for use in evaluating kingklip H&G and skinless fillets by I&J can be summarized in Table 1.

During the development of the QI scheme for hake, photographs were taken showing the above mentioned quality defects. A Microsoft Power Point presentation was prepared after a careful selection of the best pictures and then presented to a panel at random. The panel for a sensory quality grading of hake was made up of a group of people consisting of mainly I&J's staff. The outcome of the study was a hake QI scheme showing the different scores (1-6), each with a specific picture showing a particular quality parameter. The QI scheme is still being used in the wet QC (Quality Control) department to evaluate the incoming raw material (hake) by taking samples of fish bins depending on the amount of each vessel's catch (usually 10% sampling).

Table 1. The main reasons for choosing the quality parameters to be used for evaluating kingklip (*Genypterus capensis*) H&G and fillets (Per. Comm. Graz 2003).

No.	Quality defect/parameter	Reasons for choosing
1.	Discolouration	colour of the product unappealing
2.	Blood on the fat line	blemishes, usually unappealing
3.	Blood clots	blemishes, usually unappealing
4.	Gaping	fillets not intact and visually unappealing
5.	Occurrence of parasites	blemishes, usually unappealing
6.	Fillet texture	fillets not intact and visually unappealing,
7.	Skin damage	Blemishes, usually unappealing
8.	Colour of the neck end	organoleptical quality of the fish
9.	Incorrect deheading	to determine the fish yield
10.	Rugged neck	to determine the fish yield
11.	Odour	organoleptical quality of the fish

The company now wants to expand the use of the QI scheme (developed specifically for hake) in evaluating/grading other species of interest in its production, including by-catch species such as kingklip (being the main species of higher value).

Thus at the start of the current project (developing a QIM scheme for kingklip), the main aim was to develop a QI method for kingklip as per I&J's QI method for hake, using the same quality parameters and following the same procedures. However, during the study it was observed that the same QI scheme with the above mentioned quality parameters for hake are not sufficient to be used for evaluating the kingklip raw material. Therefore, the study was expanded to include organoleptic evaluation through a shelf life study as well as through the assessment of cooked fillets.

1.5. Main aims of the study

The main aim of the study is to develop a Quality Index Method (QIM) scheme (according to Barbosa and Vaz-Pires 2003) specific for raw Headed and Gutted (H&G) and skinless fillets as well as cooked fillets of kingklip (*Genypterus capensis*) using I&J's QI method for hake as a reference. Then to implement the QIM scheme to a shelf life study; i.e. to perform a shelf-life study in order to characterize the changes in freshness during storage time in ice ($0^{o}C$) using the QIM scheme developed for assessment of fresh H&G and skinless fillets, but with some modification to the scale developed by I&J for assessment of cooked fillets. Furthermore, studies of the microbial counts (Total Viable Counts (TVC), selective counts of H_2S-producing bacteria (mainly for *Clostriduim perfringens*), Coliforms and *Listeria monocytogenes*) will be done on skin and flesh samples of H&G and flesh samples of skinless fillets in order to attempt to understand the rate of spoilage during storage in ice.

2. Materials and methods

2.1. Source of fish

The study was performed at the I&J fish processing plant in Woodstock, Cape Town. A total of 94 kingklip (*Genypterus capensis*) were used: 54 fish (108 fillets) for development of the QIM scheme and 40 fish for the shelf life study of H&G and skinless fillets of kingklip. The fish were caught by I&J's own fleet of trawlers and delivered to the processing plant using insulated trucks approximately two days after capture. The fish are gutted and headed on board the fishing vessels. Thus the study for the development of a QIM scheme and shelf life study was done on H&G and fillets. The study was specifically done in an area called Primary III, a separate department within the Woodstock processing plant, where the processing of most of the by-catch species takes place.

2.2. Development of the QI scheme for kingklip

2.2.1. Procedures for development of QI scheme for fresh skinless fillets

Before starting the categorizing of defects on fresh kingklip skinless fillets, quality parameters/defects were identified through a thorough observation of the fillets in the processing flow line (Fig. 3) after the fillets were filleted by hand and skinned by trio machine. These observations were done for three weeks in March 2003 by looking at the skinless fillets in the processing flow line in order to determine what attributes are most prevalent. The following quality parameters were chosen for developing the QI scheme: Discolouration, Blood on the fat line, Blood clots/ Dark red, Gaping (weakening of the connective tissue and rupture of the fillet), Occurrence of parasites, Odour and Texture or softness of the fillet from hake QI scheme.

After selecting the above-mentioned seven quality parameters, each one was described. Scores were then ranked for each description of each parameter giving scores from 1 to 6. The higher the score in the QI scheme, the better the quality of the fillet. For any quality parameter in the QI scheme, a score of 1 is unacceptable or rejected; a score of 3 is a borderline for rejection; a score of 4 is a borderline for acceptance; and finally a score of 6 is a perfect or a good quality fish (fish product) for that parameter.

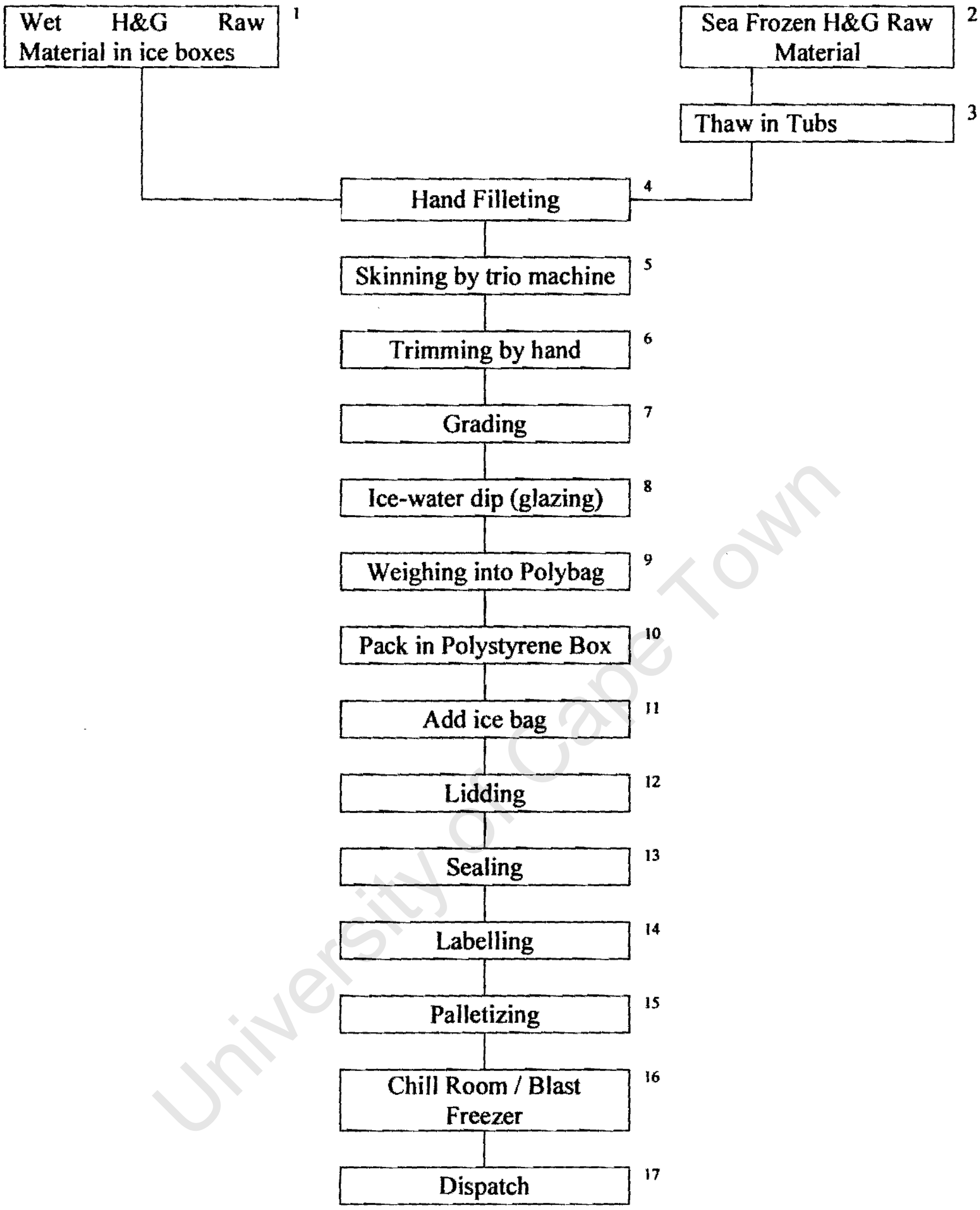


Figure 3. Process flow-line for processing chilled kingklip fillets for export.

2.2.2. Procedures for development of QI scheme for Whole and Gutted (H&G) fish

The quality parameters chosen for developing the QI scheme for grading fresh H&G kingklip skin-on were: Skin/body damage, Skin colour, Colour of the flesh at the neck end, Incorrect deheading, Texture and Odour of the fish. As mentioned above for the case of fresh skinless fillets, each of the above six quality parameters for grading H&G was fully described for scores from 1 to 6.

2.2.3. Picture record for determining quality parameters

During daily observations of the processing of kingklip in the flow lines i.e. after the fish has been filleted by hand and skinned by trio machine, a picture of that specific fillet was taken using a 35 mm slide camera (film type: Fujichrome Velvia ISO 50). A picture was taken if any of the above mentioned quality parameters for a fresh skinless fillet was noticed. The standardisation of the photographs was given priority with respect to composition and light intensity in order to insure reliable quality of photos, i.e. the camera was mounted on a tripod and was 0.5 m away from the table where the fillets were placed. A fluorescent light shining straight down into the table, an automatic speed of shutter and a flash were used during picture taking. In total for the development of QIM scheme of fresh skinless fillet, 108 pictures of skinless fillet were taken. After developing, the photographs were scanned or burned onto CD.

After careful selection of the pictures which show the quality defects in question, three Microsoft Power Point presentations each with 20 pictures were prepared for the visual quality evaluation to be performed by a panel of assessors and presented at random. Assessors were chosen among the staff of I&J on the basis of their ability and familiarity with how the scales of visual quality evaluation work. A mixture of assessors was chosen from different working fields or areas within the company (from filleters, quality control supervisors, production supervisors, management staff) to get different perceptions of assessors.

In total 30 people participated in the development and evaluation of the QI scheme of fresh skinless fillets at different times and they registered their individual responses or opinions about the quality of each picture of a fillet in the worksheet provided (Table 6 in Appendix A) for each quality parameter in the scheme. The presentations were projected into a screen using a data projector.

2.3. Application of the QIM scheme in a Shelf life study

2.3.1. Storage conditions

The H&G and skinless fillets of kingklip used in the shelf life study were obtained from I&J's trawling division. A total of 40 small H&G fish were used in the course of an independent storage trial. The mean and standard deviations of the weight of the H&G fish and skinless fillets studied were 570.22g (±114.41) and 213.35g (±55.15) respectively.

The fish were not washed with tap water because the sensorial quality of some species tends to be influenced by washing (Huidobro *et al.* 2000, 2001). Out of the total, 20 fish were filleted by hand, skinned by trio machine and packed with ice (in a plastic bag on bottom and top parts) into a clean polystyrene fish bin provided with perforated bottoms to allow drainage of melted water (always avoiding contact of the skinless fillets with the box at lower positions). The other 20 fish remained as H&G and were packed separately with flake ice into a fish bin provided with holes at the bottom for drainage (in order to prevent water from accumulating in the box). Both the fish bins were left open to allow air exchange in the boxes. The boxes were stored in a chiller (0-2 ^{o}C) until taken out for sensory and microbiological analysis as well as for photographing. Every day during the study, after taking samples for analysis, fresh ice was added to the batch of fish to maintain the ice: fish ratio throughout the storage trial.

2.3.2. Sampling plan for the shelf life study (for all analysis)

On the day of analysis, two randomly chosen fish samples (one H&G and two fillets) were removed from the batch held in ice, weighed and their raw and cooked sensory attributes were determined. A further two randomly chosen samples (one H&G and two fillets) were submitted for microbiological analysis.

Sampling was continued over an 18-day storage period (25th June 2003 to 11th July 2003). For the first three adjoining days, sampling for the shelf-life study was undertaken every day by taking four fish (two H&G and four fillets) for the sensory evaluation of fresh, cooked samples and for the microbial count study. Thereafter, sampling was undertaken every second or third day of storage time because no sampling was undertaken during the weekends. Sensory evaluation for all samples was carried out after 2, 3, 4, 7, 9, 11, 14, 16 and 18 days in ice. The sensory evaluation of raw and cooked samples were carried out in parallel along with analysis of microbial counts until 18 days of storage, but the microbial study alone was continued until 22 days (25th June 2003 to 15th July 2003) in order to follow the bacterial spoilage rate/pattern with storage time (Table 2).

Table 2. Sampling plan for the shelf life study (for all analysis)

Date	Fresh evaluation		Cooked evaluation	Microbial analysis (1 H&G and 2 fillets)
	H&G (1)	Skinless fillet (1)	Fillet (1)	
25/06/2003	*	*	*	*
26/06/2003	*	*	*	*
27/06/2003	*	*	*	*
30/06/2003	*	*	*	*
01/07/2003				*
02/07/2003	*	*	*	*
04/07/2003	*	*	*	*
07/07/2003	*	*	*	*
09/07/2003	*	*	*	*
11/07/2003	*	*	*	*
14/07/2003				*
15/07/2003				*

N.B. * denotes sampling was undertaken.

2.3.2.1. Sample preparation for sensory evaluation of raw and cooked fish

The samples for fresh evaluation (one H&G and one skinless fillet) and cooked evaluation (one fillet) were taken from the chiller (0-2 °C) and put on a small tray, covered with a polyplastic bag, packed completely with flake ice and then delivered to the experimental kitchen to be cooked and evaluated.

From a study by Sveinsdottir *et al.* (2003), it was concluded that the QIM scheme should be based upon the assessment of more than one assessor, since there are differences/variations among assessors. Further according to Hyldig and Nielsen (1997), there must be more than four assessors for validation of the QIM scheme. In addition, both Connell (1990) and Botta (1995) stated that when a panel of assessors is utilized in a QIM scheme, a maximum of six assessors is required. Therefore for this particular study, sensory analyses were performed by a panel of 5-8 assessors, all of them are I&J's staff with experience in using the sensory evaluation. They made evaluations of the fresh H&G and skinless fillets as well as the cooked flesh samples and registered their evaluation for each quality parameter in the worksheets provided (Tables 6-8 in Appendix A). Observations were carried out at room temperature using fluorescent light and with as little distraction as possible. The fresh H&G and skinless fillets were taken out of the tray and placed on a table 10-15 minutes before the evaluation. The side of the raw skinless fillet, where it was skinned, was placed facing upwards. The assessors were not informed about the storage time/day of the fish before the evaluation.

The fresh H&G and skinless fillets were evaluated using the QIM scheme developed during the study (Tables 3 and 4). Once the characteristic of a sensory attribute was determined, it was assigned a score from 1 to 6. The scores for all characteristics were then summed to give a Quality Index (QI).

Out of the seven quality parameters used for developing the QIM scheme for skinless fillets of kingklip, only four parameters were used during the shelf life study - discolouration, gaping, texture and odour. Similarly, for the case of shelf life study of H&G only the attributes of skin colour, colour of the neck end, texture and odour were used. The reasons for choosing only these parameters were that the other parameters, used in the developmental phase of the QIM scheme, did not appear to change through storage time in ice. That means there was no degree of quality deterioration throughout the storage period due to these parameters. The QIM scale used gives a score of 6 for absolutely fresh fish while decreasingly smaller scores as the fish deteriorate i.e. a score of 1 for spoiled fish. The limit of acceptance and rejection for kingklip on the QIM scheme used for the shelf-life study is a QI score of 16 and 12 out of a total of 24 scores, respectively (for both fresh H&G and skinless fillets).

Cooked skinless fillets were assessed according to the worksheet, which I&J has already developed (Table 9 in Appendix A) and used before for assessing cooked hake and other fish species. Some modifications were made in the use of scores, especially for odour (before eating) and taste/flavour (during eating), because the scores used were not consistent throughout the worksheet as they were reversed. The quality attributes used to evaluate the cooked fillets were overall flesh colour, odour, texture (before eating), texture and taste (during eating), juiciness and off tastes. The QIM scale and the scoring system used for evaluating cooked skinless filets was similar, but not the same to the QIM scale and system used in the case of raw H&G and skinless fillets. It gives a score of 5 for good quality (perfect) and a score of 1 for lowest quality of the sample, and the assessment scheme is based on a maximum score of 30. The borderline for acceptance and rejection of the cooked fillet sample is a score of 18 out of a maximum of 30 points.

In the preparation of cooked samples, small flesh portions (mostly from the loin part) of skinless fillets were prepared and wrapped in a PVC plastic bag, sealed and then steam cooked at 100 °C for 10 minutes on a stove. The scoring was carried out by putting the cooked flesh sample in a plate and it was evaluated hot (100 °C) within 5-10 minutes of cooking. The fish were judged unfit for consumption when the mean value of the QI score was less or equal to 18 out of a total of 30 points.

2.3.2.2. Sample preparation for microbial count study (microbiological analysis)

The samples for microbial analysis (one H&G and two fillets) were taken from the chiller (0-2 °C) and placed onto a sterilized polyplastic bag without contaminating them. They were delivered then to Micron Laboratory (T0136) (a division of I&J) for analysis.

For the microbiological analysis, four samples were taken. A cutting board was cleaned and rinsed with 70% ethanol in order not to contaminate the samples. Skin samples were collected by cutting skin aseptically using a sterilised knife and forceps (by dipping them into 70% ethanol and flaming them using a Bunsen burner) from every part of the H&G fish (from the loin and tail parts) in order to make a composite sample. Another two flesh samples under the skin were collected from the same H&G fish: one flesh sample from the loin part and another from the tail part. From the two skinless fillets, a composite sample of flesh was collected for analysis. For the TVC (ISO 4833), Coliforms (ISO 4832) and *Clostridium perfringens* (a selective count of H_2S-producing bacteria) (ISO 7937) analysis of the four samples, a 25 g sample was weighed into a stomacher bag containing 225 g Buffer solution (0.1% peptone water) to obtain a 10-fold dilution. For the *Listeria monocytogenes* (ISO 1129-1) analysis of the four samples, a 10 g sample was weighed into a stomacher bag containing 90 g Fraser broth solution. Blending was done in a stomacher for 1 minute and then further analysis was done by the accredited laboratory, Micron Lab T0136 using the analysis techniques mentioned above.

2.3.3. Photographs

On the day of analysis during the shelf life study, one H&G and one skinless fillet were photographed using a slide 35 mm camera (film type: Fujichrome Velvia ISO 50) before they were evaluated with the QIM scheme. Thus fish stored from 1 to 18 days in ice were photographed. The standardisation of the photographs was given priority with respect to composition and light intensity in order to insure reliable quality of photos, i.e. the camera was mounted on a tripod and was 0.5 m away from the table where the H&G and fillets were placed. A fluorescent light hanging straight above the table, an automatic speed of shutter and a flash were used during picture taking. Photographs were taken in order to show changes occurring in different quality parameters during the storage period.

2.4. Data Analysis

2.4.1. Development of the QI scheme

For each of the three presentations, the mean and standard error of the mean (SEM) of the quality score for each quality attribute, as given for each slide/picture by all assessors, were calculated. Taking one quality parameter at a time, the means of the quality scores for all slides (by combining all the three presentations) were ranked in ascending order to choose the pictures which describe the quality score from 1-6 for each parameter in question.

2.4.2. Shelf life study

From the QIM evaluation (for fresh H&G and skinless fillets as well as cooked fillets), the data for QI score and storage time in ice were analysed using linear regression techniques to determine relationships between them. The least square fit for a line, represented by the equation y = mx + b, where m is the slope and b is the intercept, was calculated for the QI score vs. storage period. The regression analysis was carried out in Microsoft Excel 2000.

A Pearson's correlation coefficient was calculated to see the degree of correlation between the QI scores (for fresh H&G and skinless fillets as well as cooked fillets) and storage time in ice.

The results from the QIM evaluation (the QI scores) were analysed in STATISTICA 6 package with one-way analysis of variance (ANOVA) to observe if a significant statistical difference of the QI score of samples (for fresh H&G and skinless fillets as well as cooked fillets) existed between storage days in ice. Furthermore, Tukey's multiple comparison test was used to determine between which storage days the significant statistical difference existed (Zar 1999).

Interaction of assessors and samples was assumed and statistical analysis was made in STATISTICA 6 package using two-factor design with interaction in the analysis of variance (ANOVA) to observe if a significant statistical difference between mean quality scores for each quality attribute, as returned by all assessors existed (Zar 1999). This is in order to test whether there is a statistically significant difference among assessors.

To observe how the individual quality attributes (for fresh H&G and skinless fillets as well as cooked fillets) deteriorate with days in ice, the mean scores as returned by all assessors were plotted against days in ice. The linear regression equation and the least square fit for a line were also calculated for the individual attributes.

For the microbial count, the log value of the TVC count was plotted against storage time in ice in order to observe the change in microbial count on skin and flesh samples of H&G (on the loin and tail parts) and flesh samples of skinless fillets with storage time in ice.

3. Results and discussion

3.1. Development of the Quality Index Method (QIM) scheme

Whenever a QIM scheme is developed for a new species, preliminary studies must be conducted to ensure that all criteria and their defined characteristics incorporated in the grade standards are appropriate and will actually be used (Hyldig and Nielsen 1997). Based on this principle, out of the seven quality attributes used for evaluation of fresh skinless fillets during developmental phase of the QI scheme, texture or softness of the fillet and occurrence of parasites were omitted from the results because the assessors were not able to evaluate them. The evaluation was done using pictures presented in a Microsoft Power Point (visual evaluation only), not using the actual fish. However, later during the shelf life study, the above mentioned quality parameters and odour of the fillet were added to the QIM scheme for fresh skinless fillets. Gaping was later omitted as well from the result, as descriptive pictures were not available from the ranked mean result of quality scores, since not enough samples of pictures were provided to the assessors for visual evaluation.

The developed QIM schemes for fresh H&G and skinless fillets, as well as cooked fillets, are included in Tables 3-5. The pictures, which are chosen from the results of the ranked mean data (Appendix C) during the evaluations using QIM scheme, showing the scores from 3-6 for each quality attribute of discolouration, blood clots and blood on the fat line, are included in Fig. 4.

After development of the QIM scheme for fresh skinless fillets, the total points available are 42 (Table 3). This describes the seven quality attributes of discolouration, blood on the fat line, blood clots, gaping, texture, occurrence of parasites and odour of the fillet. For the QIM scheme of fresh H&G, the total points available are 36 (Table 4) describing the six quality attributes of skin/body damage, skin colour, colour of flesh at the neck end, incorrect deheading, fish texture and odour. The attribute of parasite occurrence was removed from the QIM scheme for fresh H&G as no parasites were found on kingklip skin during the study period, but according to Graz (2003) there is a parasite called *Sphyrion lumpi*, which is sometimes found on kingklip skin.

Table 3. Quality Index Method (QIM) scheme developed for evaluation of fresh kingklip (*Genypterus capensis*) skinless fillets containing a ranking of the description for each parameter and the scores from 1 to 6.

Parameter	Score	Description
Discolouration	1	Excessive discolouration of the fillet
	2	Around 70% of the fillet is discoloured (aggregate area of over 10 cm^2)
	3	Moderate discoloration i.e. around 50% of the fillet is discoloured (aggregate area of >5 cm^2)
	4	Around 33% of the fillet is discoloured (discoloured aggregate area of 5 cm^2)
	5	Slight discoloration i.e. only around 20% of the fillet is discoloured (aggregate area between 2-5 cm^2)
	6	No discolouration at all (free from significant discolouration which includes bruises, browning and belly burn)
Blood on the fat line	1	Blood covers completely (100%) of the fat line
	2	Blood covers around 70% of the fat line
	3	Blood covers around 50% of the fat line
	4	Blood covers around 33% of the fat line
	5	Blood covers only 10-20 % of the fat line
	6	No blood occurrence on the fat line at all
Blood clots	1	The fillet is full of blood clots (almost 100%)
	2	Lumps or masses of clotted blood that are > 3 mm or more present in any dimension
	3	Masses of clotted blood that are equal to 3 mm present in any dimension
	4	Masses of clotted blood that are < 3 mm present in any dimension
	5	Masses of clotted blood that are between 1-2 mm present in any dimension
	6	The fillet is completely free of any blood clots
Gaping	1	Excessive gaping of the fillet (fillet of which the appearance is markedly affected due to separation of the flesh or muscle segments
	2	Around 70% of the fillet is gaped
	3	Gaping occurring almost on the loin part of the fillet (i.e. around 50% of the fillet is gaped)
	4	Around one-third (i.e. 33%) of the fillet is gaped
	5	Gaping occurring only on the tail part of the fillet (around 10-20 % of the fillet is gaped)
	6	The fillet is firm (no occurrence of gaping at all)

Table 3. Quality Index Method (QIM) scheme for skinless kingklip fillets (continued)

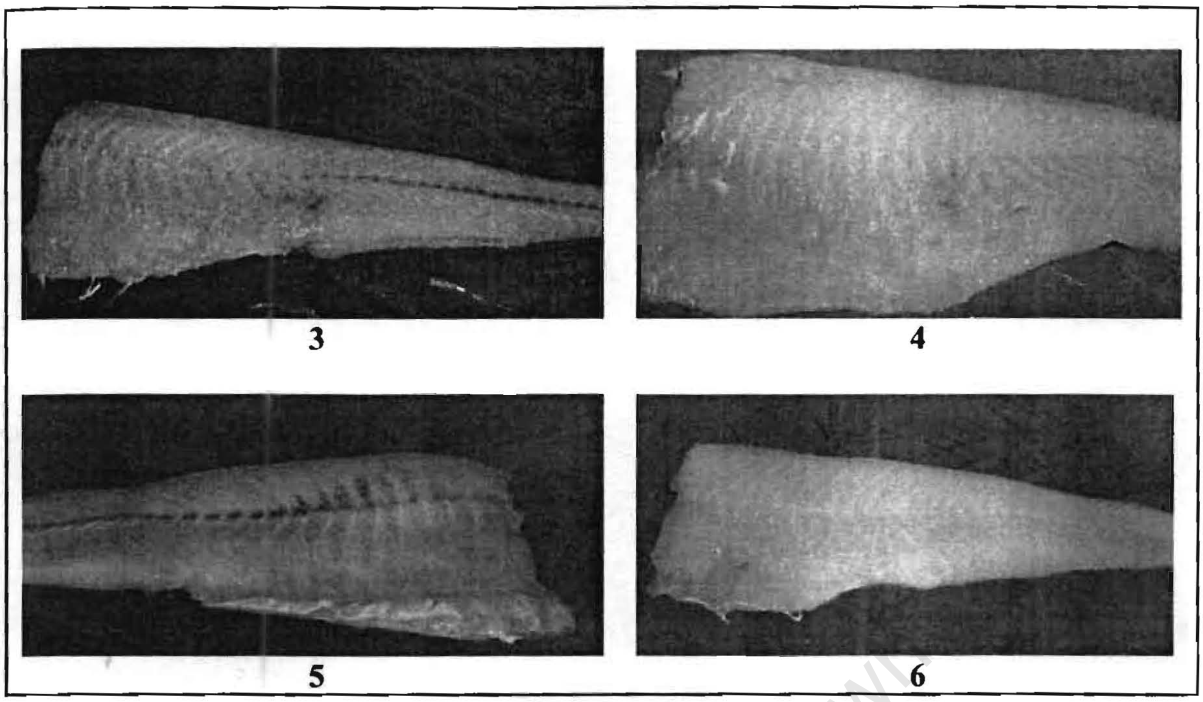
Texture/softness	1	The fillet is very soft (Pap fillet)
	2	Around 70% of the fillet is soft
	3	Around 50% of the fillet is soft
	4	Around one-third or 33% of the fillet is soft
	5	Only 10-20% of the fillet is soft
	6	The fillet is firm (no softness of the fillet at all)
Parasite occurrence	1	Parasites cover almost the whole fillet
	2	Parasites cover around 70% of the fillet
	3	Parasites cover around 50% of the fillet
	4	Two-thirds of the fillet is free from parasites
	5	Parasites cover 10-20% of the fillet
	6	The fillet is completely free from parasites
Fillet odour	1	Fillet has a very strong rancid, metallic and ammoniac smell
	2	Fillet has a strong unpleasant metallic, rancid and tainted smell
	3	Fillet has a slightly unpleasant citric and amine smell
	4	Fillet smells neutral and slightly "fishy"
	5	Loss of odour
	6	Fillet smells fresh seaweed, a smell which is only experienced in freshly caught fish
Total QIM score	**42**	

Developing principles and ideas of a total quality assurance system for the use of fresh H&G and fillets as raw materials in a fish processing industry is necessary to secure a constant rate of high quality fish (Nielsen *et al.* 1994). Therefore, the developed QIM schemes for fresh H&G and skinless fillets of kingklip will be very useful in supplying the required information on the percentage of fish, which can go to production of first grade product mainly aimed for the export market.

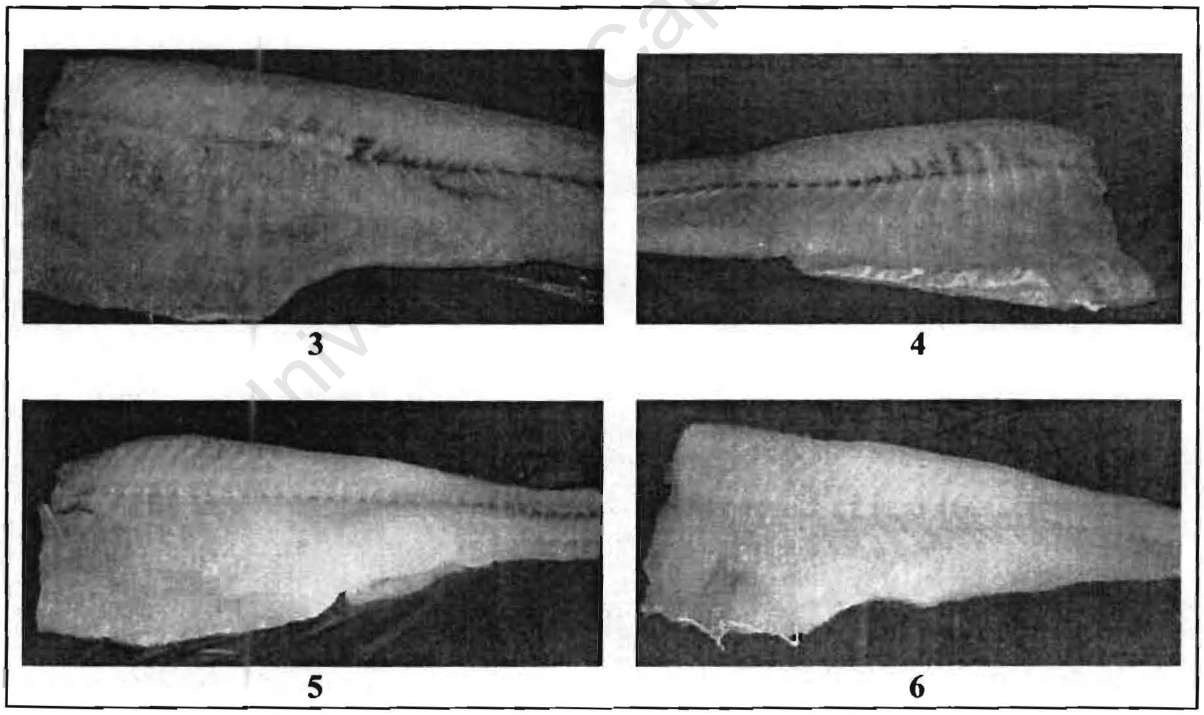
3.2. Shelf-life study

3.2.1. The QIM evaluation of raw H&G and skinless fillets as well as cooked fillets

The QIM has been considered a reliable sensory tool for assessing fish freshness in the fishery chain (Botta 1995; Nielsen 1997). The QIM scheme used in the present study for evaluating fresh H&G and skinless fillets of kingklip during the shelf-life study consisted of four criteria/attributes representing a total of 24 points.

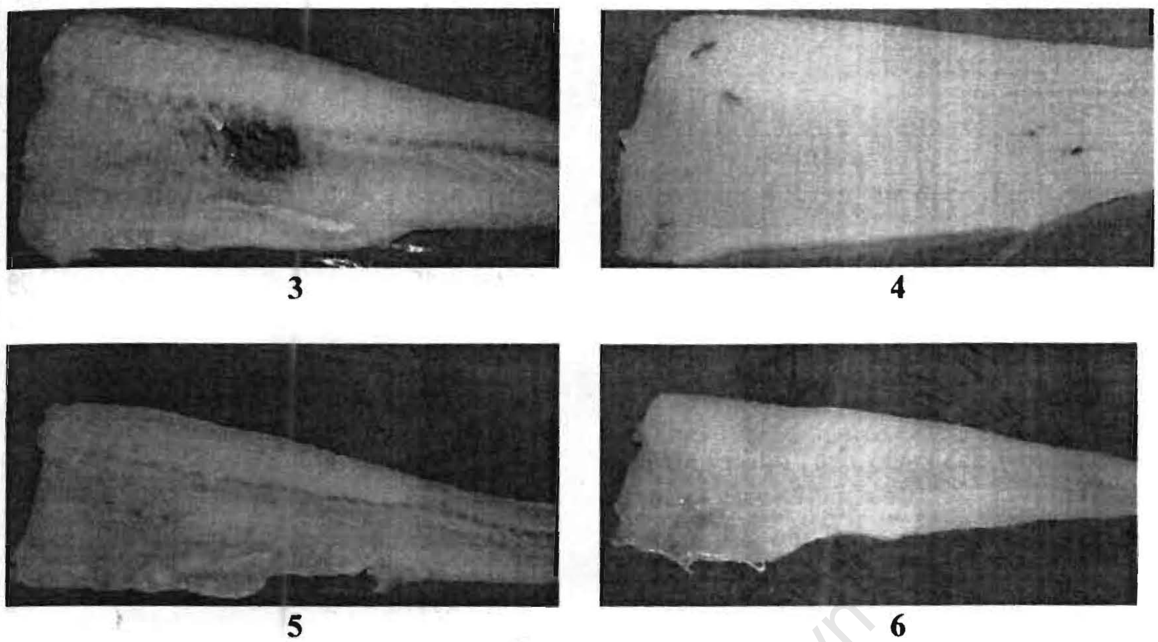


a) Pictures showing scores from 3-6 for discolouration.



b) Pictures showing scores from 3-6 for blood on the fat line.

Figure 4. Pictures showing the scores from 3-6 for attributes of discolouration, blood on the fat line and blood clots. Score value in red indicates a rejected score according the QIM scheme.



c) Pictures showing scores from 3-6 for blood clots.

Figure 4. Pictures showing the scores from 3-6 for attributes of discolouration, blood on the fat line and blood clots. Score value in red indicates a rejected score according the QIM scheme (continued).

The parameters of blood on the fat line, blood clots and occurrence of parasites for assessing fresh skinless fillets, which were originally included in the system during the developmental phase, were removed during the shelf-life study. Also the parameters of skin damage and incorrect deheading for assessing fresh H&G were removed during the shelf-life study. The reason for removing the above mentioned attributes for fresh H&G and skinless fillets was that there was no deterioration of these attributes throughout the storage period. The experimental data also indicated that the suggested QIM scheme successfully described the different freshness quality levels of iced H&G and skinless fillets of kingklip.

The sum of scores from the QIM scheme for fresh H&G and skinless fillets, as well as cooked fillets, is presented as Quality Index (QI). On the basis of the QIM scheme, it is possible to develop a calibration curve of the average QI score of the assessor's assessment against storage time in ice. They are presented in Figures 5-7.

Table 4. Quality Index Method (QIM) scheme developed for evaluation of fresh kingklip (*Genypterus capensis*) H&G containing a ranking of the description for each parameter and the scores from 1 to 6.

Parameter	Score	Description
Skin/body damage	1	Skin damage greater than 4.0 cm^2 or open flesh wounds greater than 2.0 cm^2
	2	Skin damage equal to 4.0 cm^2 or open flesh wound equal to 2.0 cm^2
	3	Skin damage between 2.0 and 4.0 cm^2 or open wound between 1.0 and 2.0 cm^2 in total area, splits longer than 2.0 cm
	4	Skin damage equal to 2.0 cm^2 or open flesh wound equal to 1.0 cm^2 in total area
	5	Skin damage less than 2.0 cm^2 or open wound less than 1.0 cm^2 in total area
	6	H&G is completely free of any skin/ body damage
Skin colour	1	The fish has completely and clearly reduced brightness and colour; the skin is dull in colour
	2	The fish has less clearly reduced brightness and colour
	3	The skin has reduced brightness and colour and it is becoming discoloured; and the fish appears rather dull
	4	The fish has reduced slightly its brightness and colour
	5	The fish has reduced less slightly its brightness and colour
	6	The colour of the skin is like newly caught fish with natural freshness of colours; it is bright with iridescent pigmentation
Colour of the flesh at the neck end	1	Excessive discolouration of the neck end; flesh in the neck end has completely changed its colour towards yellow/ brown or the fish has grey or brown discoloured neck ends such that the appearance is materially affected
	2	Around 70% of the neck end is discoloured
	3	Moderate discolouration of the neck end i.e. around 50% of the neck end is discoloured
	4	Around 33% of the neck end is discoloured; flesh in the neck end is becoming milky and somewhat translucent
	5	Slight discolouration of the neck end i.e. only around 20% of the neck end is discoloured
	6	Flesh on the neck end is completely free from any discolouration (the colour of the flesh looks clear and bright like mother-of–pearl and translucent and it is pinkish-white to off-white in colour

Table 4. Quality Index Method (QIM) scheme for evaluation of fresh H&G kingklip (continued)

Incorrect deheading	1	Fish with completely ragged neck ends
	2	Not applicable
	3	
	4	
	5	
	6	Fish cleanly and neatly cut in front of the lungs
Fish texture	1	Fish is with a very soft flesh; flesh is non-elastic and does not readily revert to its original shape if pressed by the fingers
	2	Fish is with a soft flesh
	3	Fish is with a slightly soft flesh
	4	Fish is with a less firm and less elastic flesh
	5	Fish is with a firm and elastic/ resilient flesh i.e. flesh is clearly hard and stiff
	6	Fish is with a very firm and resilient flesh i.e. flesh is very elastic and reverts to its original shape when pressed by the fingers
Fish odour	1	Fish has a very strong metallic, rancid and ammoniac smell
	2	Fish has a strong unpleasant metallic, rancid and tainted smell
	3	Fish has a slightly unpleasant metallic, rancid and tainted smell
	4	Fish smells neutral and slightly "fishy" i.e. no smell at all
	5	Fish has a loss of odour
	6	Flesh is free from any odours other than slight "sea-weedy" smell; the fish smells fresh smell of sea, a green smell
Total QIM score	**36**	

A high correlation (with a value of R^2 = 0.981 and R^2 = 0.973) between the average QI score (for fresh skinless fillets and H&G) and days of storage in ice was obtained with a slope of -0.667 and -0.839, respectively (Fig. 5 and 6). However, for the cooked fillets, a lower correlation value than that of the fresh H&G and skinless fillets, with a value of R^2 = 0.873 and a slope of -1.242, was obtained between the average QI score and storage time in ice (Fig. 7). The rejection level of mean QI score is 12 out of 24 for fresh skinless fillets and was reached at the end of shelf-life (18 days of storage in ice) (Fig. 5), but for the fresh H&G the rejection score of 12 was reached at approximately 14 days of storage (Fig. 6).

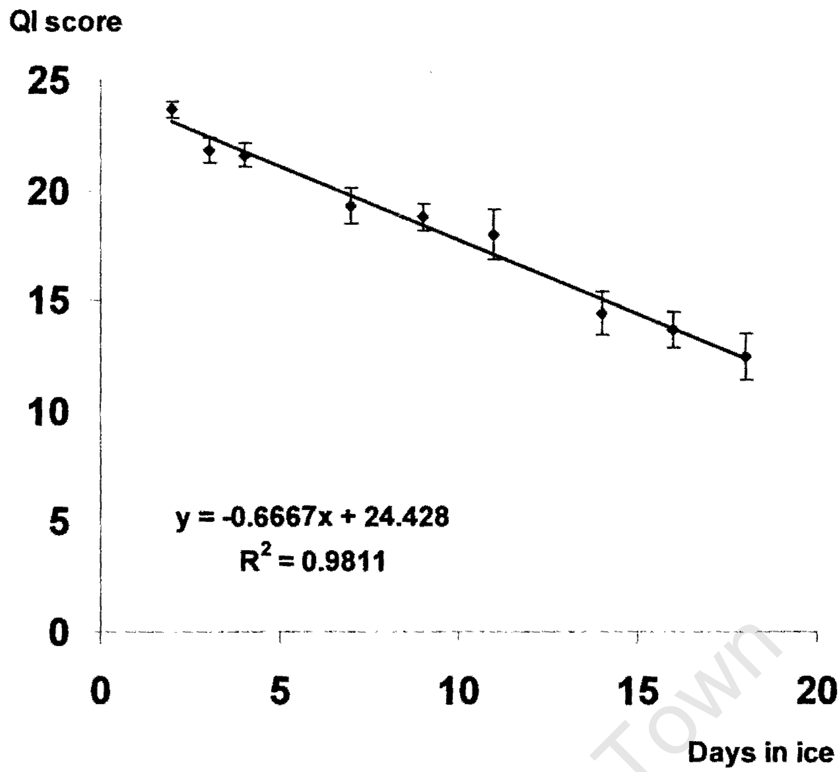


Figure 5. Average QI scores of raw kingklip skinless fillets over each day of storage analysed vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of QI score of samples as evaluated by each individual assessor.

Mean values for QI score of fresh H&G and skinless fillets reached a minimum value of 10 and 12.5 scores out of 24 at 18 days of storage in ice, respectively. The mean QI scores were found to be correlated significantly ($p < 0.05$, $n = 9$) with time of storage with a value of $r = -0.99$ for fresh skinless fillets and $r = -0.98$ for fresh H&G, which means that there is an almost perfect, negative linear relationship (Table 12a and 12b). Therefore, according to Lougovois *et al.* (2003), the QI curve could be applied to predict remaining storage life in ice and to calculate the maximum allowable number of index points to fulfil given freshness criteria when selecting any species (kingklip, in this study) for a certain type of product.

The mean values of the QI score for cooked fillets reached a minimum value of 7.17 out of 30 at 18 days of storage. It was found to be significantly correlated ($P < 0.05$, $n = 9$) with storage time ($r = - 0.93$), which means that there is a strong, negative linear relationship (Table 12c).

Table 5. Quality Index Method (QIM) scheme developed for evaluation of cooked kingklip (*Genypterus capensis*) fillet containing a ranking of the description for each parameter and the scores from 1 to 6.

Parameter	**Score**	**Description**
Colour/overall flesh colour	1	Flesh colour is dark brown and/or very discoloured with blood
	2	Flesh colour is very brownish and/or a few small blood stains
	3	Flesh colour is slightly brownish with a small blood stain
	4	Flesh colour is off-white i.e. loss of whiteness
	5	Flesh colour is white and opalescent
Odour (before eating)	1	The odour of the cooked fillet is citric, strong ammoniac
	2	The odour of the cooked fillet is slightly citric and ammoniac
	3	The odour of the cooked fillet is neutral
	4	There is loss of odour of the cooked fillet
	5	The odour of the cooked fillet is sweet, marine and seaweedy
Texture (before eating)	1	The cooked flesh falls apart completely
	2	The cooked flesh flakes very easily
	3	The cooked flesh is easily flaked
	4	There is some flaking in the cooked flesh
	5	There is no flaking of the flesh at all
Texture and Taste (during eating)	1	The texture of the cooked flesh is very soft during eating
	2	The texture of the cooked flesh is fairly soft
	3	The texture of the cooked flesh is soft/firm
	4	The texture of the cooked flesh is fairly firm
	5	The texture of the cooked flesh is firm
Juiciness (during eating)	1	The cooked flesh tastes dry during eating
	2	The cooked flesh tastes fairly dry during eating
	3	The cooked flesh tastes dry/juicy during eating
	4	The cooked flesh tastes fairly juicy
	5	The cooked flesh tastes juicy
Flavour/taste (during eating)	1	The flavour of the cooked flesh is citric, strong ammoniac
	2	The flavour of the cooked flesh is insipid, slightly citric and amine
	3	The flavour of the cooked flesh is neutral
	4	There is loss of taste and it is slightly sweet
	5	The flavour of the cooked flesh is sweet, marine and seaweedy
Total QIM score	**30**	

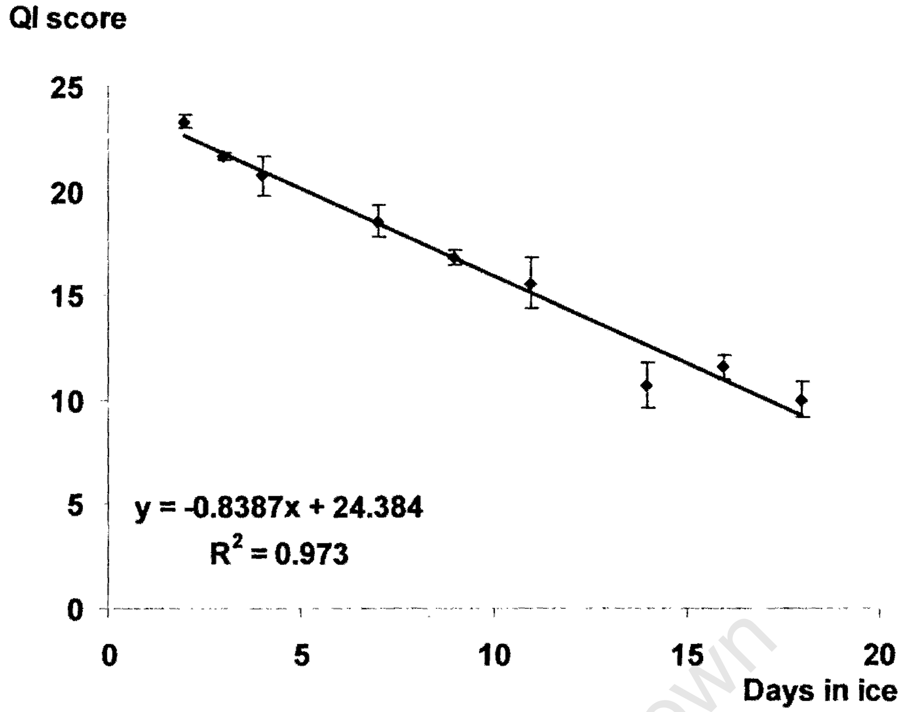


Figure 6. Average QI scores of raw kingklip H&G over each day of storage analysed vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of QI score of samples as evaluated by each individual assessor.

The difference between the mean QI score (for fresh H&G and skinless fillets as well as cooked fillets) and storage time in ice was analyzed statistically with one-way analysis of variance (ANOVA) and all were found to be significantly different ($p < 0.05$, n= 57) (Table 10). Further statistical analysis was performed using Tukey's Multiple comparison test to determine if there was a significant difference between the mean QI score from each storage day. From the results of the multiple comparison tests (Table 11), it appears that it is rather difficult to distinguish statistically between storage days in ice, especially at the beginning of the storage time. With longer storage time it becomes easier to discriminate between storage days, as the variation in sensory attributes between the individual H&G and skinless fillets of kingklip becomes more distinct. This would indicate that the individual H&G and skinless fillets of kingklip spoil at different rates. These rates could probably be caused by different position of the samples in the fish bin/box, post mortem pH or difference in fat content, which are thought to influence the spoilage rate of fish (Gram 1995b).

According to Nielsen (1995b) and Hyldig and Nielsen (1997, 2001), the progress of deterioration in the sensory quality of raw fish stored in ice can be divided into four phases:

- **Phase one:** The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic and the sweet taste is maximized 2-3 days after catching.
- **Phase two:** There is a loss of the characteristic odour and taste. The flesh becomes neutral but has no off-flavours and the texture is still pleasant.
- **Phase three:** There is sign of spoilage and a range of volatile, unpleasant smelling substances are produced, depending on the fish species and type of spoilage (aerobic and anaerobic). One of the volatile compounds may be trimethylamine (TMA) derived from the bacterial reduction of trimethylamineoxide (TMAO). TMA has a very characteristic "fishy" smell.
- **Phase four:** During this phase sickly sweet, cabbage-like, ammoniacal, sulphurous and rancid smell develops and the texture becomes either soft and watery or tough and dry. The fish can be characterized as spoiled and putrid.

The evaluation method used for assessing fresh kingklip H&G and skinless fillets in this study describes a decline in sensory quality, which can be divided into four phases similar to those above. From Figures 5 and 6, we can distinctly observe the four phases: phase one extending from the catch day until 2.5 days of storage in ice (0 ^{o}C); phase two until 5.5 days of storage; phase three until 13 days; and phase four until the end of shelf life study (18 days in ice). In phase four, where the curve for QI score of fresh H&G (Fig. 6) goes below rejection level of 12 out of 24, the fish is characterized as no longer suitable for human consumption.

Mean QI scores of cooked kingklip fillets over time is shown in Fig. 7. The deterioration of sensory quality of the cooked fillets was faster than that of fresh H&G and skinless fillets, with a slope of -1.242 and also faster than that reported before for ungutted whole, iced gilthead sea bream with a slope -0.304 (Kyrana *et al.* 1997).

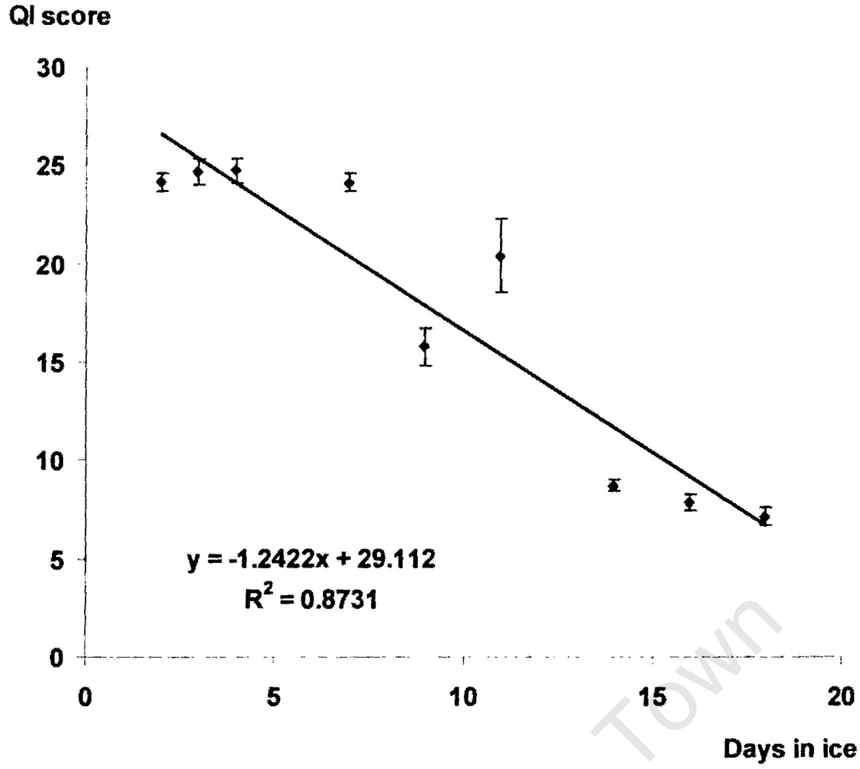


Figure 7. Average QI scores of cooked kingklip fillets over each day of storage analysed vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of QI score of samples as evaluated by each individual assessor.

During the first seven days of storage, there was a fresh, sweet, marine and seaweedy odour and flavour characteristics of the species until there was a loss in the intrinsic fresh odours and flavours by day nine. The flesh then became insipid and flavourless by about 10-12 days and slight sour, citric, ammoniac and rotten off-odours and off-flavours were evident by 14-15 days. Finally, the flesh became unpalatable with strong citric, ammoniac off-flavours and off-odours by day 18 of storage. Such fish is not acceptable for human consumption and was rejected as spoiled and putrid by the assessors after 14 days of storage and the sensory assessment was not continued further, especially tasting evaluation, due to the strong citric and ammoniac odour. Similar results were found by a study on iced gilthead sea bream (*Sparus aurata*) (Lougovois *et al.* 2003). From Fig. 7, the rejection score of 15 out of 30 for cooked fillets was reached at approximately 13 days of storage.

The average QI score for cooked fillets almost stayed constant (ca. mean QI of 24) with some slight variation for the first seven days of storage in ice; decreased to a score of 15.8 by day nine; increased to a score of 20.4 by day 11; then finally decreased until a minimum value of 7.17 score by day 18 (the end of the storage time in ice). This fluctuation in the average QI score could be due to variation among assessors.

Using the QIM scheme in estimating the freshness of fish gives the assessors the opportunity to choose between score points ranging from 1-6 for fresh H&G and skinless fillets or from 1-5 for cooked fillets. Occasionally, rather large deviation from the correlation line is observed, especially in the case of QI scores for cooked kingklip fillets. Analyzing the data further, it appears that the individual kingklip are somewhat different; obtaining different scores. This could be due to biological variation among individual fish sampled for different days of storage. The difference in the QI score could also be partly caused by the different positions of the fish samples in the bins or different nutritional status/stress of the fish before fishing (Sveinsdottir *et al.* 2002).

The QIM curve does not follow the traditionally accepted S-curved pattern for deterioration of chilled fish during storage, because then it will not be possible to distinguish between fish at the start and at the end of the plateau phase (Jonsdottir 1992). A system with a linear relationship makes it possible to distinguish between fish at the start of the plateau phase and fish near the end of the plateau phase (Jonsdottir 1992). The linear response with time of storage is a great advantage for a processor or a purchaser who would require to receive the fish at an earlier stage, when there is still some time to process and package it before the quality is unacceptable (Bremner 1985).

To discuss the freshness assessment of fish in general, freshness quality grading to determine seafood freshness is becoming widely used within the seafood industry. It may be used by commercial seafood companies to ensure that the product will meet the expectations of both buyers and regulatory agencies, seafood buyers to ensure that the product being purchased is meeting their expectations, and seafood regulatory agencies to confirm that the seafood being produced is meeting their regulations (Botta 1995).

Since seafood quality grading involves the use of both a structured category scale and trained assessors, the procedure can be considered objective (Larmond 1986, as cited in Botta 1995). This is because, in addition to the assessors functioning as analytical instruments, the words (employed to define the respective score points of the particular quality attribute) describe specific sensory properties (Connell and Howgate 1986, as cited in Botta 1995). Therefore, the criticism that freshness quality grading (since it is based on sensory evaluation) is subjective, causing the results to be extremely variable and of limited use, is not valid (Larmond 1986, as cited in Botta 1995).

In general, sensory methods are the most acceptable and widely used in the fishing industry, but their disadvantages are that they are dependent on the person applying them, and a period of training is demanded (Jonsdottir 1992). A method, which is very expressive and independent of the persons who are involved, is of interest in the fishing industry. This is one of the reasons why many people have been looking for a rapid and direct reading of the freshness that could be operated in the fish industry and at the market place. Some of the methods which have been tried are chemical, e.g. TMA (trimethylamine) and K-value, physical, e.g. pH, "Fish tester" value, "Torrymeter" readings, and "RT-Freshness Grader" readings (Jonsdottir 1992). Most of the chemical methods depend on the measurement of one of a large number of complex changes that take place when fish lose their freshness and begin to spoil (Damaglou 1979, as cited in Jonsdottir 1992). The disadvantages of these methods are that they require laboratory equipment and are destructive. In fact they are more useful in controlling spoilage than in measuring freshness of fish (Jonsdottir 1992). The physical methods are based on measuring the dielectric properties and they have the advantage of being non-destructive and fast (Jonsdottir 1992). Additional advantages of using the electrical tester include ease of use, immediate response, portability, and minimal training requirements (Lougovois *et al.* 2003), but their disadvantages are that the readings for one species can depend on season, fat content and fishing ground (Martinsdottir 1987, as cited in Jonsdottir 1992). Out of the different methods for assessing fish freshness mentioned above, the most acceptable and widely used in the fishing sector are sensory methods, especially the QIM scheme.

According to an experiment by Jonsdottir (1992), chemical methods were found to be unreliable in the first 70% of the storage time compared with the QIM method. Therefore, by using the QIM scheme, the fish processing companies can be able to document the quality control procedure for raw material and also to use the information about the quality index in the production planning. The implementation of the QIM scheme has demonstrated its applicability through a successful adoption to a quality control system (Jonsdottir 1992).

3.2.2. Performance of individual assessors

Variation observed in QI scores from different assessors is shown in Figures 8 and 9. The difference in the QI scores given by each assessor appears to increase with storage time, indicating that the assessors are more consistent in analysing very fresh kingklip H&G and skinless fillets than fish of lesser freshness. From Figures 8 and 9, it can be seen that individual assessors participating in the QIM evaluation usually tended to score either higher or lower than average QI scores throughout the storage time.

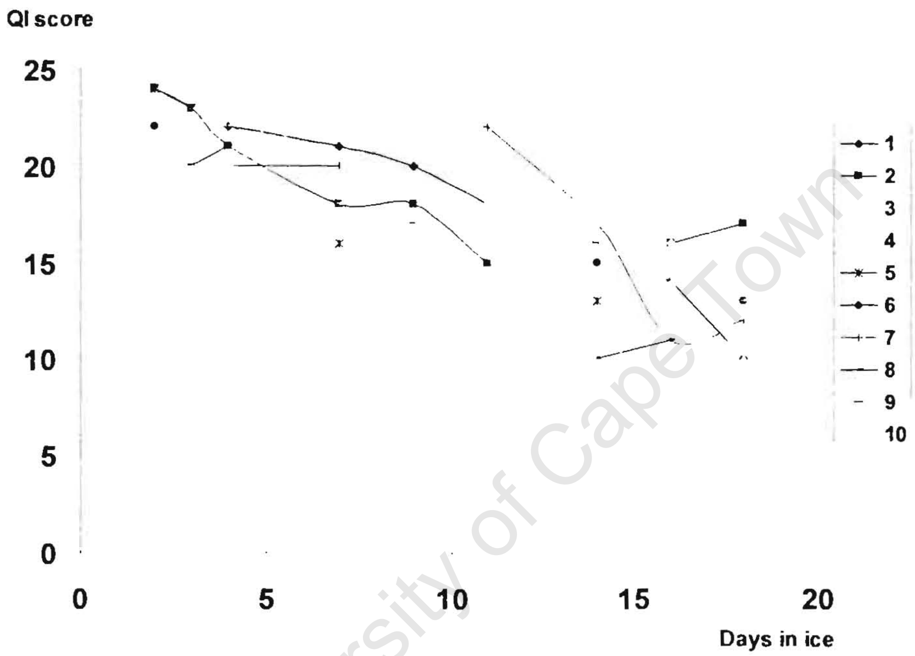


Figure 8. Average QI score of fresh skinless kingklip fillets stored in ice each day of analysis, as given by each assessor.

In the case of QI scores for cooked fillets (Fig. 10), the scores from each assessor seem to follow the same decreasing pattern with storage time. indicating that the assessors are in agreement in evaluating the cooked kingklip fillet samples than the fresh H&G and skinless fillet samples, even though some variation in evaluation still exists among the assessors. The variation was greatest early in the storage period but decreased with storage time in ice. This indicates that the assessors became more unanimous in their evaluation of cooked kingklip fillets as decay progressed, which may be because changes in cooked fillets became more prominent with time.

The variation in QI scores indicates that not all of the assessors are fully qualified in sensory evaluation using QIM schemes. Thus more training before the evaluation could have reduced the variation among assessors. This further emphasises the importance of training and material in the form of photographs and guidelines.

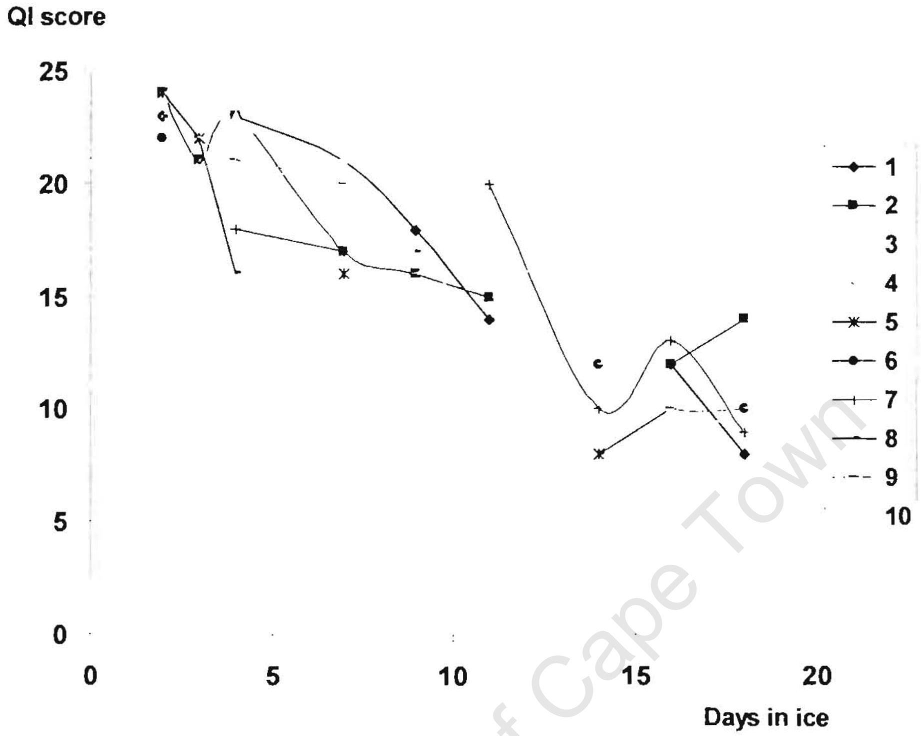


Figure 9. Average QI score of fresh kingklip H&G stored in ice each day of analysis, as given by each assessor.

Furthermore, there could be some variation among the assessors in the way they evaluate the samples, especially the cooked samples, in relation to their cultural background. Among the ten assessors involved in evaluating the cooked fillet samples, eight are coloured and two are white and this combination of people from different cultural or racial background could have caused variation in the results of sensory evaluation (Per. Comm. Graz 2003). Especially in the first days of storage, we observe great variation among assessors in the QI scores as shown in Fig. 10, but with further increase in storage time, all of the assessors seem to agree in their evaluation.

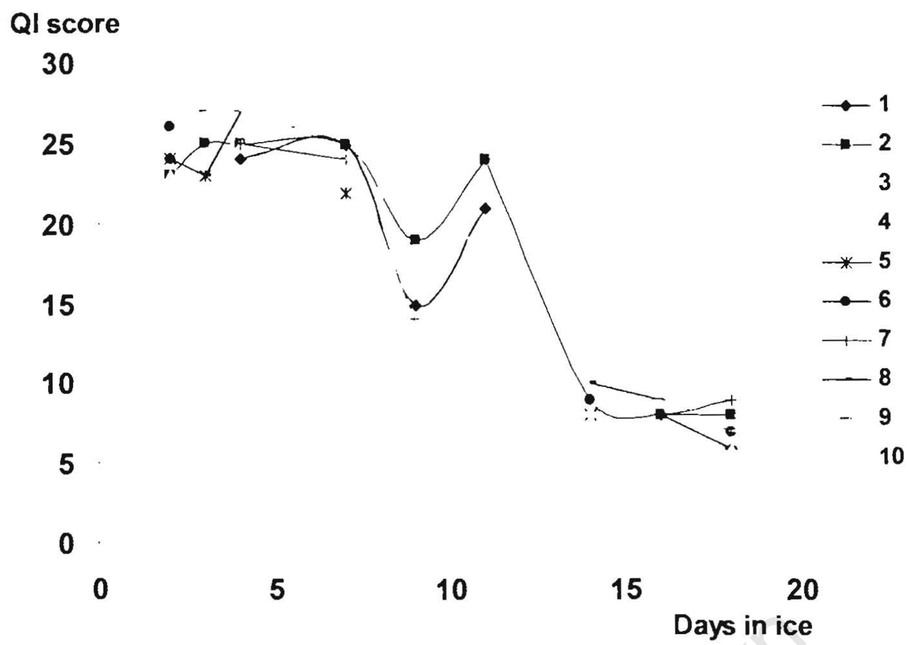


Figure 10. Average QI score of cooked kingklip fillets stored in ice each day of analysis, as given by each assessor.

During sensory evaluation with the QIM, to test if a significant difference existed between quality scores as given by each assessor for each quality attribute assessed with storage time, analysis was performed using two-factor design with interaction in the analysis of variance (ANOVA) assuming that there is interaction between assessors and samples. The results showed that there is a statistically significant difference between assessors involved in assessments (Table 13). This supports the idea that the individual assessors participating in the QIM evaluation of kingklip performed differently and there is variability in their responses. Therefore, the freshness assessment with the QIM scheme should be based upon the assessment of more than one assessor and all should be well-trained in order to reduce the variation.

3.2.3. Changes occurring in individual quality attribute

It was assumed in the QIM scheme that the scores for all attributes decrease with storage time. The decrease was observed in this study for the QIM schemes of fresh H&G and skinless fillets as well as cooked fillets, but to a different extent for different quality attributes. To observe how the scores for the different quality attributes decrease with storage time, the mean scores were plotted vs. days in ice (Figures 11-13).

3.2.3.1. Quality attributes for fresh kingklip skinless fillets

The average scores of all attributes for fresh kingklip skinless fillets as given by all assessors decrease with storage time in ice. Changes occurring in each one of the quality attributes are discussed below.

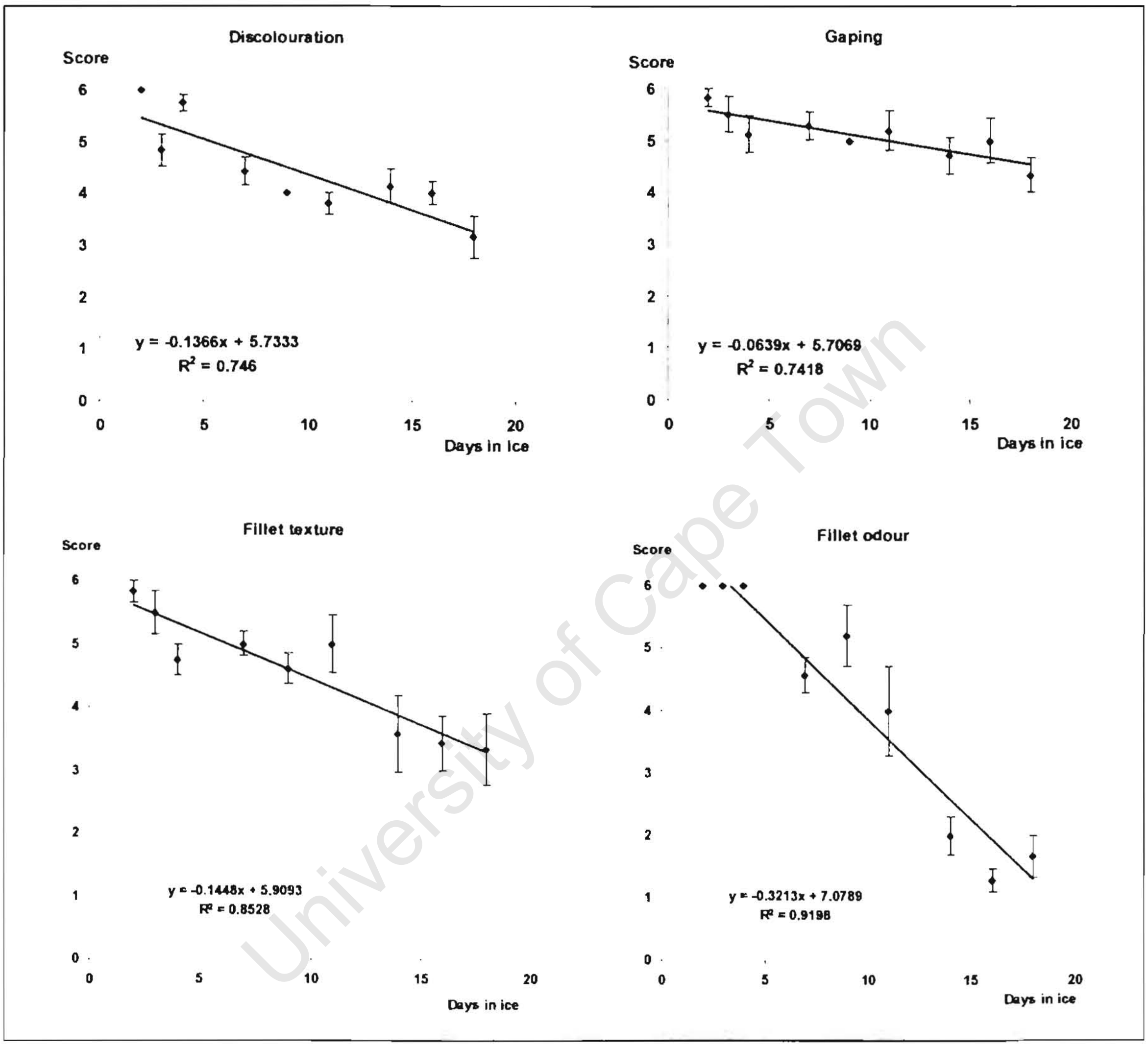


Figure 11. Average scores of quality attributes for fresh kingklip skinless fillets vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of the quality score given by each individual assessor.

The average score for discolouration was six at storage day two (start of the shelf-life study) as the fillet was still fresh as described in the QIM scheme (Table 3) with no discolouration at all. However, with increase in storage days the fillets became more discoloured and the average score reached 3.17 at the end of storage time (18 days in ice) with an aggregate discoloured area of greater than 5 cm^2 and it was rejected. The estimation of fillet colour appears to be rather difficult as Fig. 10 indicates, since fillet stored four and fourteen days in ice received higher scores than fillet stored three and eleven days in ice, respectively. The reasons could be due to individual differences between the fillets and the position of the fillets in the fish bin as different fillets were sampled randomly from different positions in the fish bin for different days of storage. The average score of discolouration did not reach a rejection level after 18 days of storage.

The average scores for gaping of fresh skinless fillets decrease with storage time, but the rate of decrease was not as rapid as that of discolouration (i.e. slope for gaping curve and discolouration curve are -0.0639 and -0.1366, respectively). The average score for gaping was 5.83 at two days of storage as the fillet was still firm with no gaping at all. The decrease in score could probably be due to variation among individual assessors. The mean score then decreased to an average of 4.33 after 18 days of storage with one-third of the fillet gaping, but still acceptable, indicating that there is no degradation of the connective tissues in the fillet. Here also, as the case for discolouration, the estimation of gaping appears to be rather difficult as Fig. 11 indicates, since fillet stored seven and sixteen days in ice received higher scores than fillet stored four and fourteen days in ice respectively. The average score of gaping would not lead to rejection after 18 days of storage.

The average score for fillet texture decreased with time, but the decrease was steeper with a slope of -0.145 than that of discolouration and gaping, indicating that there is a degradation of the flesh (autolysis) not the connective tissues in the muscles. The fillet was very firm, fresh and in rigor at two days of storage with an average score of 5.83 and this decrease in score could probably be due to variation among individual assessors. However, the resolution of rigor causes the muscle to relax again and the fillet becomes limp during storage in ice, but no longer as elastic as before rigor. The fillet becomes soft probably due to autolysis influenced by both fish muscle enzymes and microbial enzymes (Nielsen 1995b; Gill 1995). The average score decreased to 3.33 after 18 days of storage with around 50% of the fillet becoming soft, and reached a rejection level at the end of the shelf life. Similarly as is the case for discolouration and gaping, estimation of fillet texture also appears to be rather difficult as Fig. 11 indicates, since fillets stored seven and eleven days had higher scores than fillets stored four and nine days, respectively. The average score of fillet texture was found to be better correlated (with a value of R^2 = 0.853) with days of storage than the average scores for discolouration and gaping (Fig. 11).

The average score for fillet odour decreased constantly with storage time. The decrease was very rapid with a slope of -0.321, compared to the other attributes evaluated. At the beginning of the storage time when the fillet was still fresh, the average score remained six (perfect) until four days of storage and the odour was described as fresh seaweedy, which is experienced only in freshly caught fish. This is probably because fresh fish contains low levels of volatile compounds, which contribute fresh like odours (Olafsdottir and Fleurence 1997). The sour and ammoniacal odour of fillets after 18 days of storage was probably caused by microbial activity, e.g. short chain acids, alcohols, sulphur compounds and amines (Olafsdottir and Fleurence 1997). The average score decreased to 1.67, which is below the rejection level. From Fig. 11, the rejection level of fillet odour was reached at approximately 12.5 days of storage.

The average score of fillet odour was found to be more highly correlated (R^2 = 0.92) with storage time than the other three quality attributes for evaluation of fresh kingklip skinless fillets. As Fig. 11 indicates, fillet stored nine days received higher score than fillet stored seven days. Therefore, estimation of fillet odour becomes rather difficult as is mentioned above for the other three attributes, and the reasons for difficulty in estimation for all attributes used in evaluation of raw skinless fillets could be due to individual differences between kingklip fillets, as different fillets were sampled for different days of storage. The position of the fillets in the bin with regard to the oxidation reaction also could be a reason for the difficulty in estimation, especially in the case of fillet odour.

3.2.3.2. Quality attributes for fresh kingklip H&G

The average scores of all attributes for fresh kingklip H&G as returned by all assessors decreased with storage in ice. Changes occurring in each one of the attributes assessed are discussed below.

The average score for skin colour was 5.83 at two days of storage when the fish was still fresh and this decrease in score could probably be due to variation among individual assessors. The skin colour was described in the QIM scheme (Table 4) as like newly caught fish with natural freshness of colours and bright with iridescent pigmentation. The average score decreased to 2.83 after 18 days of storage, which is below the rejection level. At this stage the fish had less clearly reduced brightness and colour. From Fig. 12, the average score reached a rejection level at approximately 16.5 days of storage and the estimation of skin colour seemed to be rather difficult since kingklip stored four, nine and sixteen days received higher scores than kingklip stored three, seven and fourteen days respectively. The average score of skin colour was found to be highly correlated with storage time in ice (with a value of $R^2 = 0.945$) compared to the other three attributes for evaluation of fresh H&G.

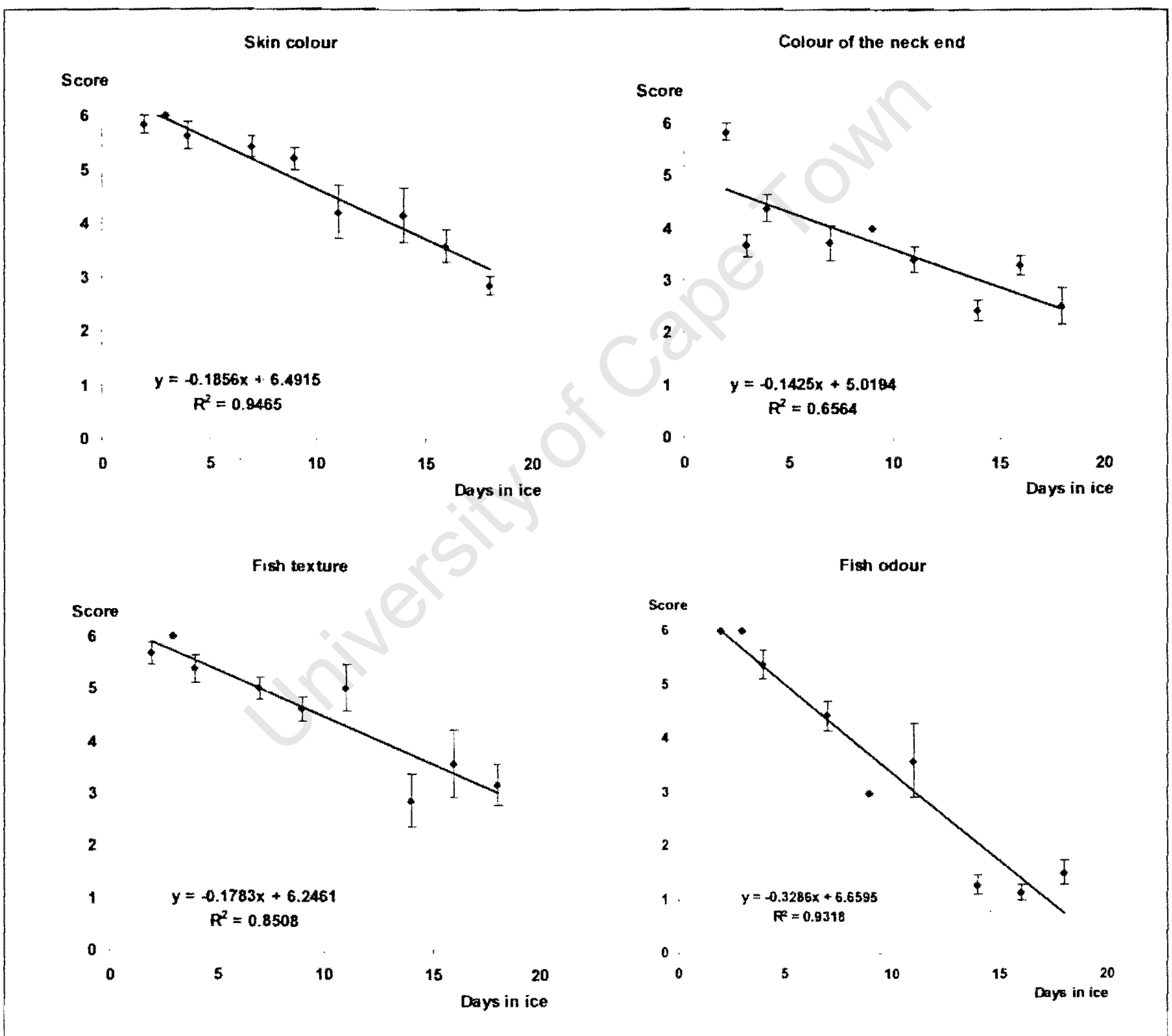


Figure 12. Average scores of quality attributes for fresh H&G kingklip vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of the quality score given by each individual assessor.

The average score for colour of the flesh at the neck end was 5.83 at two days of storage when the fish was still fresh and its colour was described as bright like mother-of-pearl, translucent (pinkish white in colour) and the flesh looked clear. This decrease in score could probably be due to variation among individual assessors. The average score then decreased to a minimum value of 2.5 at the end of shelf life with 70% of the flesh becoming discoloured. The colour changed towards yellow/brown due to oxidation reaction of the flesh (Per. Comm. Graz 2003). From Fig. 12, the score reached a rejection level of three at approximately 12 days of storage and kingkilp stored four, nine and sixteen days in ice received a higher score than kingklip stored three, seven and fourteen days, respectively.

Texture is an important parameter that changes with storage time in ice (at 0^oC), involving the stiffness in rigor and softening of the fish flesh during storage (Sveinsdottir *et al.* 2002). The average score for fish texture was 5.67 at two days of storage while the fish was still fresh and in rigor, and it was described in the QIM scheme (Table 4) as fish with a very firm and resilient flesh. The decrease in score could be due to variation among assessors and the fish could have been stored on board for more than two days. At this stage the flesh is very elastic and reverts to its original shape when pressed by the fingers. As discussed for fillet texture, the resolution of rigor causes the muscle to relax again and through storage in ice the fish becomes soft due to autolysis (Nielsen 1995b). Then the average score decreased to a minimum value of 3.17 at the end of storage life with a slightly soft flesh and it is rejected. As is evident from Fig. 12, kingklip stored three, eleven and sixteen days in ice received higher scores than kingklip stored two, nine and fourteen days respectively.

The average score for fish odour decreased constantly with storage time similar to the average score for fillet odour, but the decrease was rapid with a slope of -0.329 as compared to the attributes of skin colour, colour of the neck end and fish texture (with slopes of -0.186, -0.143, -0.178, respectively). This indicates that there was higher microbial degradation resulting in development of off-odours. The average score remained constant with a value of six until three days of storage and the fish was still fresh with a seaweedy smell (as found with fillet odour) and this could be due to low levels of volatile compounds, which contribute fresh like odours in raw fish. The score then decreased to a minimum value of 1.5 with a strong metallic, rancid and ammoniac smell. From Fig. 12 the rejection level was reached at approximately 12 days of storage.

The average score of fish odour was found to be more correlated ($R^2 = 0.93$) with storage time than the other three quality attributes for evaluation of fresh H&G. In this case, kingklip stored eleven days received higher scores than kingklip stored nine days as shown in Fig. 12. Therefore estimation of fish odour seems to be difficult (similar to what has been discussed for the other three attributes for fresh H&G) and the reason for difficulty in estimation for all attributes used in the evaluation of fresh H&G could be due to individual differences between kingklip H&G as different fish were samples for different days of storage.

3.2.3.3. Quality attributes for cooked kingklip fillets

Cooked fish flavour is an excellent indicator of freshness quality and may provide precise information on the time of storage following harvest. However, especially trained assessors are required for objective sensory evaluation (Botta 1995) and this can be both expensive and inconvenient (Connell 1995).

Out of the seven attributes used for evaluating cooked fillets, only off-odour before eating and off-taste during eating constantly decreased with time in ice with $R^2 = 0.757$ and $R^2 = 0.540$ with slopes of -0.299 and -0.275, respectively (Fig. 13). The sensory score for flavour of the cooked fillets decreased with storage time and the fresh flavour characteristic of the species was strong for 2-4 days, then slowly decreasing in intensity to a relatively flavourless stage by 10-12 days. Off-flavours, probably due to bacterial metabolites or due to autolysis, were evident by 13-15 days and as spoilage progressed, the off-flavours increased in intensity and became more evident until the fish became unpalatable by about 18 days.

During the first part of storage life (until seven days) (Fig. 13), there was a constant quality score of five for the cooked fillets with a characteristic flavour and odour of the species. It then decreased abruptly to a quality score of one by day nine for both attributes. The scores increased again to 3.4 by day 11 for both attributes, probably due to variation among assessors during evaluation or due to the position of the fillets in the bin. Finally the value for odour decreased again to a score of one at day 14 and remained the same until day 18 of storage in ice (the end of shelf life). The quality score for flavour of the cooked fillets was not evaluated by the assessors after day 14 of storage, since it developed a sour and ammoniac off-flavour and was not palatable.

For routine freshness evaluations, the QIM scheme could be a more effective sensory tool, as it is faster, non-destructive and requires less training than sensory evaluation of cooked fish flavour. Using the quality index, the post-mortem age of iced fish could be predicted to within less than two days, which would be an adequate measure for quality management purposes in the fishery chain (Lougovois *et al.* 2003).

3.3. Photographs

During the shelf life study, photographs of H&G and skinless fillets of kingklip were taken on the day of analysis before they were evaluated with QIM scheme. The main aim of taking pictures was to show the changes occurring during the storage time of the different quality attributes included in the QIM scheme, especially the appearance. Fig. 14 shows some examples of photographs demonstrating different freshness stages during the shelf life study.

The changes occurring in the outer appearance of H&G and skinless fillets with storage time in ice are clearly demonstrated in Fig. 14. The colour of the skin changes from being pearl shiny (with iridescent pigmentation) and darker pinkish brown to a faded and very dull pinkish colour. The colour of the flesh at the neck end of the H&G fish changes from clear, bright and translucent towards yellow/brown in colour. The colour of the fillet changes from being pearl-shiny with no discolouration to excessive discolouration. All these changes are listed in the QIM scheme for fresh H&G and skinless fillets (Tables 3 and 4).

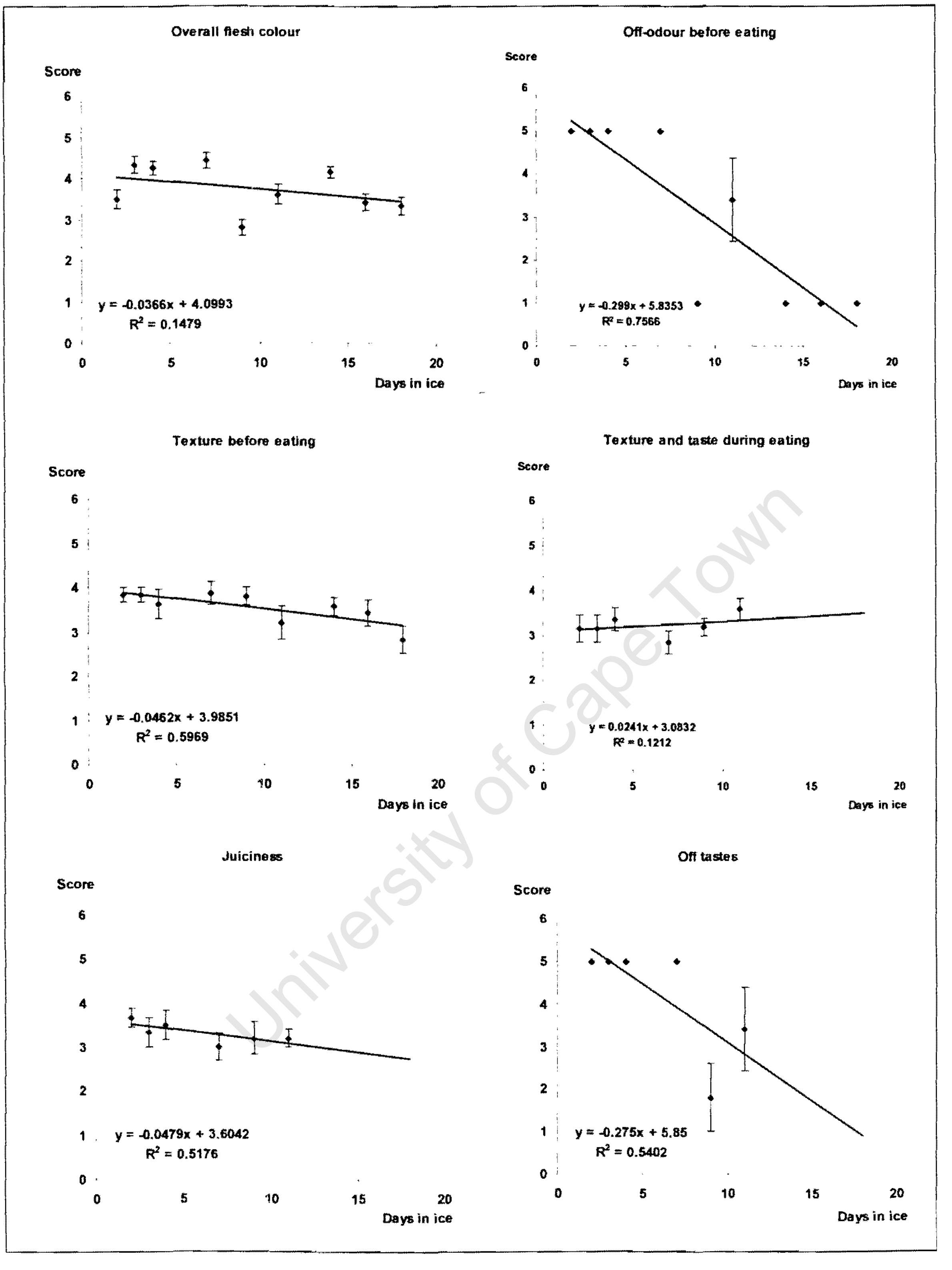
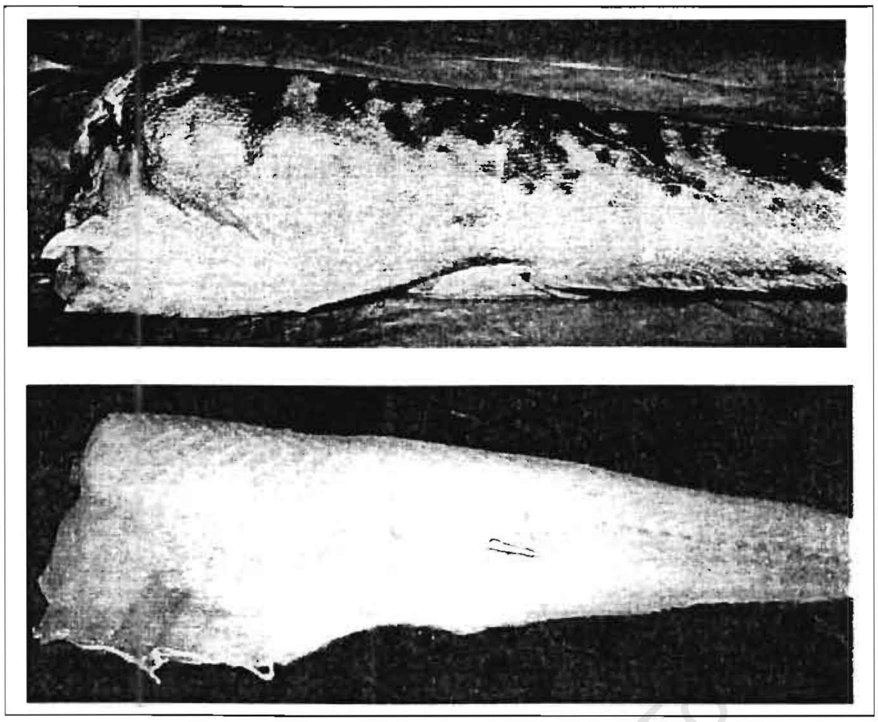
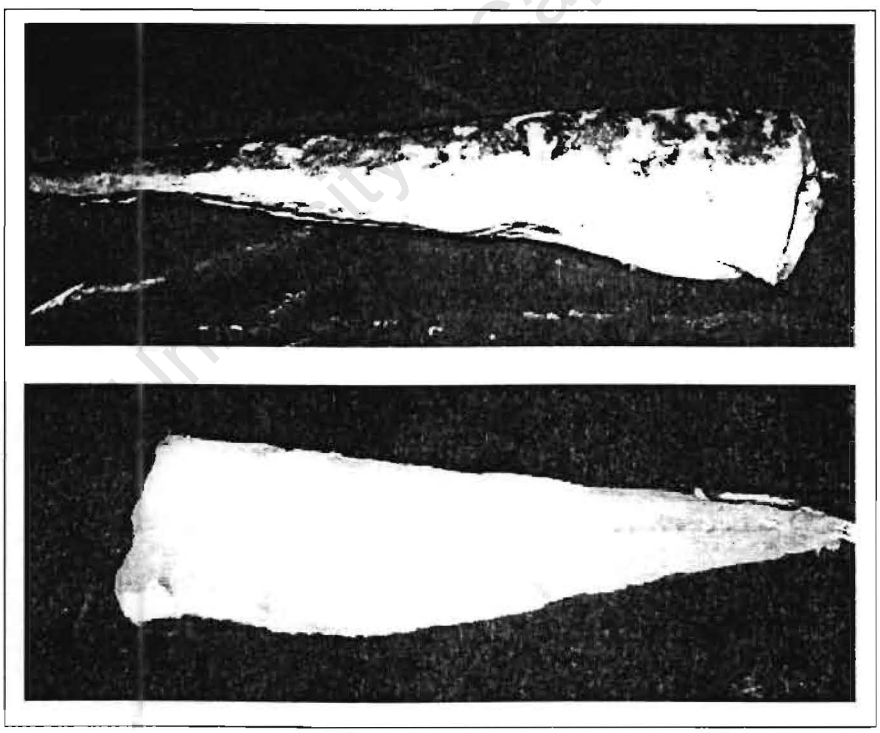


Figure 13. Average scores of quality attributes for cooked skinless kingklip fillets vs. days in ice. Each point represents the mean with Standard Error of the Mean (SEM) of the quality score given by each individual assessor.



a) Kingklip H&G and skinless fillet stored two days in ice.



b) Kingklip H&G and skinless fillet stored 18 days in ice.

Figure 14. Photographs of kingklip H&G and skinless fillets at two different freshness stages: at the beginning of shelf life study (two days in ice), and at rejection limits (18 days in ice).

3.4. Microbial count

Fig. 15 shows the results of Total Viable Count (TVC) on kingklip skin and flesh samples (from the loin and tail parts) of H&G and flesh samples of skinless fillets during storage.

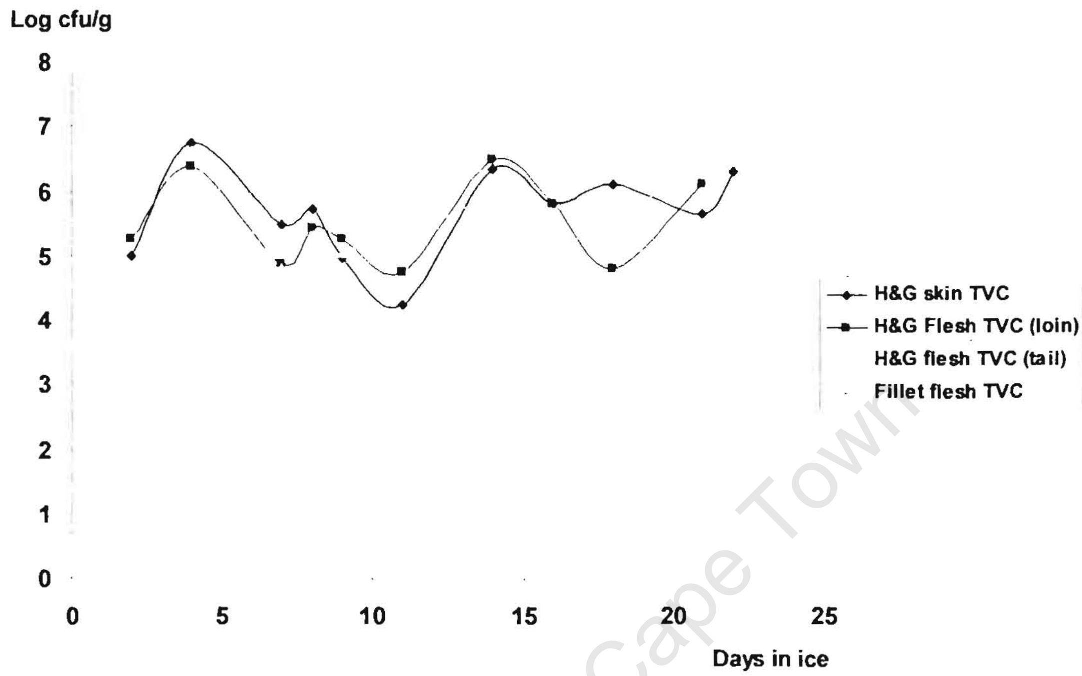


Figure 15. Log cfu/g (Total Viable Count (TVC)) on skin and flesh of H&G and flesh of skinless fillets for kingklip stored in ice. Each point represents the average of duplicate analysis.

At the beginning of the shelf life study (two days of storage time), the TVC count on skin of H&G and flesh sample of skinless fillets were ca. 10^4 cfu/g. This can be considered normal for newly caught fish (Liston 1980, as cited in Sveinsdottir *et al.* 2002). Also according to Olafsdottir *et al.* (1997), newly caught fish contain a diverse microflora and TVC of 10^2 to 10^6 cfu/g are usual on whole fish and cut fillets. After 22 days of storage at 0 °C, the TVC count on skin of kingklip H&G was found to be only ca. 10^6 cfu/g.

The TVC count on flesh of H&G (from the loin and tail parts) at the start of shelf life study was ca. 10^5 cfu/g, which is higher than the TVC count on skin of H&G and fillet sample (both at 10^4 cfu/g). After 22 days of storage, the TVC count on flesh of H&G still remained 10^5 cfu/g after having many fluctuating values, which is lower compared to the TVC on skin and fillet samples (10^6 cfu/g). Similar results were found in a study made on farmed Atlantic salmon, *Salmo salar* (Sveinsdottir *et al.* 2002), where the TVC count on skin was ca. 10^3 cfu/cm^2 at the beginning of the storage time and increased to ca. 10^8 cfu/cm^2 after about 20 days of storage in ice.

The TVC count on kingklip skin and flesh samples of H&G as well as flesh samples of skinless fillets did not exceed spoilage levels of 10^7 cfu/g (IFST 1999, as cited in Lougovois *et al.* 2003), which are commonly found on fish, until after 22 days of storage reaching only 10^6 cfu/g for skin samples of H&G and flesh samples of skinless fillet and 10^5 cfu/g for flesh samples from the loin and tail parts of H&G (Fig. 15). As bacterial loads in the flesh samples of H&G and fillet remained rather low ($<10^5$ cfu/g) until day seven, it was assumed that the early loss of flavour resulted primarily from autolytic reactions and thus the exclusion of bacteria from the fresh kingklip would not prevent the product from becoming less acceptable.

Off-odours and off-flavours are the main symptoms of spoilage and their development frequently coincides with the presence of high microbial numbers. It is usually assumed that the two factors are related and that bacterial counts in the order of 10^7 cfu/g or higher are necessary to induce the production of off-odours and off-flavours (Jay 1986, as cited in Ravn-Jorgensen *et al.* 1988). However, in this study the TVC counts on skin and flesh samples of kingklip did not reach 10^7 cfu/g even after 22 days of storage, but off-odours and off-flavours became prevalent after 14 days of storage. The reason for this could probably be that kingklip may develop inhibitors against bacterial growth (Per. Comm. Graz 2003), but this requires further investigation.

Magnusson *et al.* (1996, as cited in Sveinsdottir *et al.* 2002) counted TVC and H_2S producing bacteria in farmed salmon, where the TVC reached ca. 10^8 cfu/cm^2 after 19 days in ice on the skin, but ca. 10^5 cfu/g in the flesh after the same storage time in ice. This is in agreement with the results obtained in this study especially for the case of TVC count on flesh of kingklip H&G, but for the case of TVC count on skin the results found in this study are very much lower than those of Magnusson *et al.* (1996).

The TVC count in flesh samples of kingklip H&G at the rejection limits (at 18 days of storage) observed in this study are considerably lower than is usual at the rejection limits. According to Olafsdottir *et al.* (1997), the TVC of fish products are typically 10^7-10^8 cfu/g at the point of sensory rejection and this could be caused by the high amounts of

H_2S producing bacteria at the end of the storage time, as they are probably responsible for spoilage (Capell *et al.* 1997). This supports the rejection of the cooked kingklip fillet samples after ca. 14 days of storage as the TVC count could probably be dominated by H_2S producing bacteria at that time. A study by Sveinsdottir *et al.* (2002) on farmed Atlantic salmon (*Salmo salar*) found very few H_2S producing microbes as part of the microflora at the beginning (<10 cfu/cm^2), but their share of the total viable count increased with storage time. The TVC (mainly H_2S producing microbes) on salmon skin was found to be ca. 10^8 cfu/cm^2 after about 20 days of storage in ice, which is very much higher than found on skin samples of kingklip after 22 days of storage (10^6 cfu/g).

Fig. 15 shows there are three stages/phases in the bacterial growth curve (especially for the case of H&G skin TVC curve) (Prescott *et al.* 1996). These are:

1. **Exponential growth or log phase**: this is a stage where there is an exponential increase in the number of bacteria until it reaches the maximum carrying capacity, and log numbers of TVC counts plotted against time is linear.
2. **Inhibition/competition or decline phase**: a stage where there is a fall in bacterial numbers from the maximum carrying capacity mainly due to space and nutrient limitation, as the bacteria require additional space and nutrient for further growth.
3. **Degradation phase**: a stage where the skin starts to degrade very much and the number of bacteria starts to increase, since they have acquired more space and nutrient for further growth by penetrating deeply into the skin.

During the degradation stage, bacteria or the enzymes they secrete are able to penetrate the skin deeply and invade the flesh by moving between the muscle fibres and degrade tissue components to produce the unpleasant odours and flavours associated with spoilage (Hobbs 1982; Howgate 1982; Connell 1990; Gram 1995a).

There is a stationary stage (maximum carrying capacity) after the exponential stage where the TVC count remained constant, but it is very short and the curve falls abruptly into the inhibition stage (Fig. 15). In the case of H&G flesh TVC (loin parts) curve, we can observe that there is the same pattern of bacterial growth as the case of H&G skin TVC curve, but we can distinctly see that there is an additional second inhibition stage and this could probably be caused by the inclusion of flesh from the neck ends in the loin samples for the TVC analysis. For the case of H&G flesh TVC (tail parts) curve, the same pattern of bacterial growth curve with storage time is observed as in H&G skin TVC curve.

In the curve of fillet flesh TVC, there is a delay in the exponential growth stage, known as log phase, of bacterial numbers and the maximum number of bacterial growth (carrying capacity) occurs later (after nine days of storage) than the case for H&G skin and flesh TVC curve, where the peak is reached after four days. This delay could be as a result of the freezing effect from the trio machine to the upper most part of the fillet during the skinning process. The process of freezing could kill or damage the bacteria (Per. Comm. Graz 2003). The changes brought about by processing will determine which types of bacteria continue to grow and also remove some bacteria, kill others and add other species to the fish (Hobbs 1982). Therefore, the bacterial population takes longer to recover from the shock of the change of the environment and to adapt to the new environment, and that is why the peak point is reached after nine days of storage.

In this study, selective counts for *Clostridium perfringens* (being an indicator of the H_2S producing bacteria) gave negative results in all samples, but still the bacterium *Shewanella putrefaciens* could probably be responsible for producing H_2S, which is the cause for fish spoilage by developing off-odours and off-flavours, the symptoms of spoilage. Test/analysis for *S. putrefaciens* was not possible because the Micron Laboratory (T0136) does not have the specific media for growing the bacteria and the specific instrument (fluorometer) to detect and enumerate it. More recently, the bacterium *S. putrefaciens*, which is one of the most predominant bacteria associated with spoilage of fish products by producing hydrogen sulphide, was determined as the specific spoilage organism (SSO) of some chilled fresh fish (Olafsdottir *et al.* 1997). This microorganism can be enumerated in iron-containing agar, and correlation coefficients as high as -0.97 were achieved when comparing log numbers of *S. putrefaciens* with the remaining storage life of aerobically stored fish, as determined by sensory evaluation irrespective of whether or not that organism is itself causing the rejection (Gram *et al.* 1987; Capell *et al.* 1997). Owing to the selection of microorganisms in chilled fish, the correlation between SSO and freshness is usually higher than between TVC and freshness (Ravn-Jorgensen *et al.* 1988).

Sulphide producing bacteria (SPB) and species of *Pseudomonas* are commonly identified with spoilage of aerobically stored fish (Huss 1995). Sulphide producers are important in generating offensive, fishy, rotten and H_2S-off-odours and off-flavours associated with the spoilage of marine, temperate water fish stored in melting ice, the predominant SPB being *Shewanella putrefaciens* (Gram 1992; Gram *et al.* 1987). In warmer waters, *Pseudomonas* spp. can be the dominant spoilage bacteria (Koutsoumanis and Nychas 2000, as cited in Lougovois *et al.* 2003), however in this study, no counts were made on *Pseudomonas* spp. Counts of sulphide-producers have been used as indicators of iced fish spoilage (Ravn-Jorgensen *et al.* 1988), and though they constitute a small proportion of the total aerobic flora, they provide a useful indicator of quality deterioration and could be used to determine the time to rejection (Lougovois *et al.* 2003).

From the microbial analysis made on Coliforms and *Listeria monocytogenes*, the results were all negative. The aim in making microbial analysis for these types of bacteria was to evaluate the hygienic quality of the kingklip H&G and skinless fillets during handing and processing. The results indicate that the process does not appear to add extra species of bacteria to the fish.

Microbiological methods of assessing freshness are useful for research or product development but are not practical for routine use, as they require expensive laboratory equipment and trained staff, are destructive, and can be labour intensive (Lougovois *et al.* 2003). Therefore, a fast and reliable method such as QIM scheme is necessary for assuring freshness specification of the starting material and making sure that the product will not become stale when distributed and displayed (Lougovois *et al.* 2003).

Since bacterial activity is the prime cause of spoilage in unfrozen fish, measurement of bacterial numbers would provide a direct index of freshness. Unfortunately, there are both practical and theoretical objections to this approach. Bacteriological tests generally are time consuming i.e. they require 2-3 days to complete, some types of test even longer. Clearly this is a serious disadvantage in the quality control of wet fish. Also not all species of bacteria present on fish cause spoilage, and as spoilage proceeds the number of spoilage organisms as a proportion of the total bacterial population changes and the number of bacteria able to grow at low temperature increases. Hence a count of the total number of bacteria is only an approximate measure of the numbers of the relevant organisms and may be misleading as an index of freshness (Howgate 1982). Furthermore, Huss et al. (1974) stated that TVC count is a very poor indicator of both quality and remaining shelf life of chilled fish. Thus TVCs in seafood correlate poorly with the degree of freshness or remaining shelf life (Delgaard 2000).

4. Conclusion and Recommendation

In this project, a QIM scheme for fresh H&G and skinless fillets as well as cooked fillets of kingklip (*Genypterus capensis*) was developed in a shelf life study. The scores for quality attributes included in the QIM schemes for fresh H&G and skinless filets as well as cooked fillets decreased somewhat differently with storage time in ice, but the scores given by all assessors added together as a Quality Index (QI) gave a negative linear relationship between QI scores and storage time in ice. The correlation between mean QI scores for fresh skinless fillets and H&G and days in ice was almost perfect, negative and linear with a value of $r = -0.99$ and $r = -0.98$ respectively. However, in the QI of cooked fillets, the correlation was found to be strong, negative and linear with a value of $r = -0.93$. The linear relationships between the QI score (y) for raw skinless fillets and H&G as well as cooked fillets and storage time in ice (x) were found to be the formulas:

1. $y = -0.6667x + 24.43$
2. $y = -0.8387x + 24.38$
3. $y = -1.2422x + 29.11$, respectively.

The least square fit for a line was represented by the equation $y = mx + b$, where m is the slope and b is the intercept of the curve. From a statistical analysis with one-way ANOVA, the QI scores for fresh H&G and skinless fillets as well as cooked fillets were significantly different with storage time ($p < 0.05$, $n = 57$).

The high correlation found between the QI scores and storage time in ice makes it possible to predict the past storage time in ice. As the maximum storage time of kingklip in ice has been determined at 18 days, this information can be utilized directly for assessment with the QIM scheme for kingklip to determine the remaining storage time in ice. The precise and descriptive QIM schemes developed for fresh H&G and skinless fillets of kingklip, supported by photographs showing visible changes occurring during storage time in ice for each attribute, will make it easy in future to assess the freshness of kingklip. Furthermore, it will provide more valuable and reliable information about the freshness quality of kingklip so that evaluating kingklip raw material will greatly help in production planning and quality management of the products made from kingklip by I&J. Therefore, the QIM schemes for kingklip may become very useful in producing first grade product by monitoring the performance of the fishing vessels by giving them feedbacks concerning the quality of their catch and making recommendations, which may initiate better handling on board and improve fish quality.

In continuation of this project, it will be important to repeat the shelf life study of kingklip using more samples of fish to observe if similar slopes for the linear regression between the QI scores and storage time in ice will be obtained. Furthermore, during storage in a chiller, kingklip may have undergone some temperature fluctuations even though they were covered with flake ice, as it was evident during the weekends because the machine was turned off. Therefore, it would of interest to examine how kingklip that has undergone temperature fluctuations during the storage time in a chiller would fit to the QIM scheme.

The assessors participating in the sensory evaluation during the shelf-life study of kingklip with the QIM scheme performed differently, as some of them gave higher or lower scores throughout the storage time. Using two-factor design with interaction in ANOVA and assuming that there was interaction between assessors and samples, a significant difference was found among assessors ($p < 0.05$, $n = 224$ (for fresh H&G), $n = 228$ (for fresh skinless fillets), $n = 342$ (for cooked fillets)) indicating that all performed differently. More training may have reduced the difference in assessor performance, but it is very difficult to have a sensory evaluation panel perform in precisely the same way even if the assessors are all well trained.

The descriptions given in the QIM scheme are very precise and accurately describe the changes occurring in outer appearance, odour and texture of fresh kingklip H&G and skinless fillets as well as cooked fillets, facilitating the freshness assessment making the individual performance differences as small as possible. Using photographs of H&G and fillets of kingklip during sensory evaluation with the QIM scheme may support the assessment even further. These methods together make it possible to evaluate the freshness of kingklip in a fast and reliable way, providing reliable information about its quality and remaining shelf life in ice. However, the unavoidable chance of differences among assessors, as it is observed in the study, implies that the freshness assessment with the QIM scheme should preferably be based upon the assessment of more than one assessor and all should be well trained in order to reduce the variation.

Based upon the sensory evaluation of fresh H&G and skinless fillets as well as cooked fillets, the maximum storage life of kingklip has been determined as 18 days in ice, but taking the palatability of the cooked fish into account, the shelf life was found to be 14 days, since after that time the assessors cannot make the tasting evaluation of the cooked fish. The quality of the cooked kingklip fillets did not change much through the storage time until day 12-13. However, by day 14 the changes in odour and flavour became more evident and the off-odours and off-flavours increased significantly until 18 days of storage. Differences among panelists were evident for all evaluated attributes in the QIM scheme for cooked fillets and this might be reduced with more efficient training aimed at more harmonized assessment.

From the study, it can be concluded that the same QI scheme with the same quality parameters, as used for sensory evaluation of hake by I&J, cannot be used for evaluation of kingklip since following the same QI scheme is not enough to supply the required information on the percentage of fish which can go to production of first grade product aimed primarily for export markets. Therefore, organoleptic assessments should always be included in the QIM scheme, to be used for evaluation of kingklip raw material, so that the scheme can be used in allocating the raw material into different products and produce first grade product based on the quality grading system.

The total viable count (TVC) were low at the beginning of the storage time (ca. 10^4 cfu/g on skin samples of H&G and flesh samples of skinless fillets, but ca. 10^5 cfu/g on flesh samples of H&G (from the loin and tail parts)). This is within the range considered to be normal for a newly caught fish. However, at the end of the shelf-life (18 days in ice), TVC counts had only reached ca. 10^6 cfu/g on skin samples of H&G and flesh samples of skinless fillets and ca. 10^5 cfu/g on flesh samples of H&G (from the loin and tail parts). This TVC count on skin of H&G kingklip is still low compared to a similar study on farmed Atlantic salmon, *Salmo salar* where the TVC count on skin of salmon was found to be ca. 10^8 cfu/cm^2, but the TVC count on flesh of H&G is of the same level as the TVC count on flesh of salmon, ca. 10^5 cfu/g.

From previous studies, off-odours and off-flavours are the main symptoms of spoilage and their development coincides with high microbial numbers (of the order of 10^7 cfu/g or higher). However, from this study the TVC counts from all samples of kingklip did not reach that level even after 22 days of storage, but still development of off-odours and off-flavours became evident only after 14 days. Thus it can be concluded that development of symptoms of spoilage in kingklip stored in ice do not coincide with a value of TVC count of 10^7 cfu/g, but they are found to coincide with a value of 10^6 cfu/g. The reason for this could probably be that kingklip may develop inhibitors against bacterial growth. However, this requires further investigation.

As bacterial loads in the flesh samples of H&G and skinless fillets of kingklip remained rather low (ca. 10^5 cfu/g) until day seven of storage in ice, the early loss of flavour and odour was assumed to be as a result of autolytic reactions. Thus exclusion of bacteria from the fresh kingklip would not prevent the product from becoming less acceptable by consumers.

QIM schemes for evaluating the quality of fresh H&G and skinless fillets as well as cooked fillets are developed and implemented in a shelf life study of kingklip during this study, but further study should be made in order to develop QIM schemes for evaluating frozen H&G and fillets since I&J also uses sea frozen kingklip H&G as a raw material for its production in addition to fresh kingklip raw material. Thus developing a QI scheme for frozen kingklip will greatly help in production of good quality products.

The results from the microbial count could have shown a clear pattern in the TVC curves if more replicate samples of skin and flesh of H&G and flesh of fillets were used during the shelf life study. But due to the availability and value of kingklip and also time limit, this was not possible. Therefore, in future studies in order to see a clear pattern in the increase of bacterial numbers with storage time, studies of microbial count should be performed by taking more replicate samples of skin and flesh of H&G and fillets.

Studies on selective counts of hydrogen-sulphide producing bacteria were only made on *Clostridium perfringens* and were found to be absent, but there is a great possibility that *Shewanella putrefaciens* could be one of the hydrogen-sulphide producing bacteria responsible for the production of off-odours and off-flavours, symptoms of spoilage in kingklip. Thus in future, studies on testing microbial counts of *S. putrefaciens* should be made on skin and flesh samples of kingklip in order to prove its presence (whether it is responsible for producing sulphide off-odours and off-flavours in kingklip). Furthermore, from the microbial count made on Coliforms and *Listeria monocytogenes*, they were found to be absent in all samples tested. Therefore, from these results we can conclude that the process does not appear to add extra species of bacteria to the fish, although this requires further investigation.

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Appendix A: Worksheets

Table 6. Worksheet used for evaluation of the picture/slides of fresh skinless fillets during the development phase of the QIM scheme by the assessors.

Voyages 1-3

Date:.................

A worksheet to be filled by a sensory quality evaluation panel for a study on: **Development of a Quality Index (QI) scheme for kingklip (Genypterus capensis).** Please evaluate the pictures of fresh skinless fillets from the slide presentation according the quality attributes mentioned and indicate your reply on the spaces provided below using scores 1-6.

Name:...................................

Slide No.	Quality parameters/ defects				
	Discolouration	Blood on the fat line	Blood clots/ Dark red	Gaping	Occurrence of parasites
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

*** Thank you for your cooperation!***

Table 7. Worksheet used for evaluation of fresh H&G during a shelf life study.

Product: Kingklip (*Genypterus capensis*)

Date: ________________

Name: __________________________

Please evaluate the sample (H&G) according to the following quality parameters/ defects using the QIM scale (from 1-6).

	Quality parameter	QIM Scales					
No.	Quality Parameter	1	2	3	4	5	6
1.	Skin / body damage						
2.	Skin colour						
3.	Colour of the neck end						
4.	Incorrect deheading						
5.	Texture of the fish						
6.	Occurrence of the parasite						
7.	Odour of the fish						

Comments (Please state anything you observe to be quite odd i.e. other than the above mentioned quality defects)

*** Thank you for your cooperation!***

Table 8. Worksheet used for evaluation of fresh fillets during a shelf life study.

Product: Kingklip (*Genypterus capensis*

Date: _______________

Name: ___________________________

Please evaluate the sample (fillet) according to the following quality parameters or defects using the QIM scale (from 1-6)

No.	**Quality parameter**	**QIM Scales**					
	Scale	**1**	**2**	**3**	**4**	**5**	**6**
1	Discoloration						
2	Blood on the fat line						
3	Blood clots						
4	Gaping						
5	Texture or softness						
6	Occurrence of parasite						
7	Fillet odour						

Comments (Please state anything you observe to be quite odd i.e. other than the above mentioned quality defects)

*** Thank you for your cooperation!***

Table 9. Worksheet used for evaluation of cooked kingklip fillets during a shelf life study.

Product: Kingklip (*Genypterus capensis*
Date: ______________
Name: __________________________

*** Please rinse your mouth with water before tasting the sample***

You have received a kingklip sample. Please evaluate it according to the following attributes in the order presented and indicate your choice in the numbered blocks provided.

Colour/ overall flesh colour

1	2	3	4	5
Dark brown	Brownish	Slightly brownish	Off-white	White

Odour (before eating)

Do you detect any foreign or off-odours? (Please specify)

Yes	No	

Texture (before eating)

Flakiness (ease of flaking/breaking apart of flesh)

1	2	3	4	5
Falls apart	Very easily	Easily flaked	Some flaking	No flaking

Texture and Taste (during eating)

Texture

1	2	3	4	5
Soft	Fairly soft	Soft/Firm	Fairly firm	Firm

Juiciness

1	2	3	4	5
Dry	Fairly dry	Dry/Juicy	Fairly juicy	Juicy

Do you detect any foreign or off-tastes? (Please specify)

Yes	No	

Comments (Please state anything you observe to be quite odd i.e. other than the above mentioned quality defects)

____
____
____
____

*** Thank you for your cooperation!***

Appendix B: Statistical results

Table 10. Results of one-way analysis of variance (ANOVA) to test if a significant statistical difference between QI scores (for fresh H&G and skinless fillets as well as cooked fillet) existed with storage time in ice (0°C).

a) One-way ANOVA between QI scores for fresh H&G with storage time in ice.

Variable	Analysis of variance Marked effects are significant at $p < 0.05$							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	P
QI scores H&G	1227.011	8	159.6264	197.0238	48	4.1046	38.8890	0.0000*

b) One-way ANOVA between QI scores for fresh skinless fillets with storage time in ice.

Variable	Analysis of variance Marked effects are significant at $p < 0.05$							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	P
QI scores fillet	797.9641	8	99.7455	190.9131	48	3.9773	25.0784	0.0000*

c) One-way ANOVA between QI scores for cooked fillets with storage time in ice.

Variable	Analysis of variance Marked effects are significant at $p < 0.05$							
	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	P
QI scores cooked fillet	3192.497	8	399.0622	157.6429	48	3.2842	121.5087	0.0000*

N.B. * denotes that it is statistically significant at $p < 0.05$.

Table 11. Average Quality Index (QI) of samples (of fresh H&G and skinless fillets as well as cooked fillets) of each group analyzed (kingklip stored in ice). One-way analysis of variance (ANOVA) with Tukey's Multiple comparison test used to estimate if the groups were significantly different from each other.

a) Average QI of fresh H&G samples

Group (storage time in ice)	Mean (QI score)	Not significantly different from group/groups
2	23.33	3,4
3	21.67	2,4,7
4	20.75	2,3,7,9
7	18.57	3,4,9,11
9	16.80	4,7,11
11	15.60	7,9,16
14	10.71	16,18
16	11.57	11,14,18
18	10.00	14,16

b) Average QI of fresh skinless fillet samples

Group (storage time in ice)	Mean (QI score)	Not significantly different from group/groups
2	23.67	3,4
3	21.83	2,4,7,9,11
4	21.63	2,3,7,9,11
7	19.29	3,4,9,11
9	18.80	3,4,7,11
11	18.00	3,4,7,9,14
14	14.43	11,16,18
16	13.71	14,18
18	12.50	14,16

c) Average QI of cooked fillet samples

Group (storage time in ice)	Mean (QI score)	Not significantly different from group/groups
2	24.17	3,4,7
3	24.67	2,4,7
4	24.75	2,3,7
7	24.14	2,3,4
9	15.80	--
11	20.40	--
14	8.71	16,18
16	7.85	14,18
18	1.17	14,16

Table 12. Results of Pearson's correlation coefficient calculated to see the degree of correlation between the QI scores (for fresh H&G and skinless fillets as well as cooked fillets) and storage time in ice.

a) Correlation between QI scores for fresh H&G and storage time in ice.

Var. X & Var. Y	Correlations Marked correlations are significant at $p < 0.05$ (Casewise deletion of missing data)										
	Mean	Std. Dv.	r(X, Y)	r^2	t	p	N	Constant dep: Y	Slope dep: Y	Constant dep: X	Slope dep: X
Days in ice	9.3333	5.8309									
QI score H&G	16.5562	4.9576	-0.986	0.973	- 15.891	0.0000*	9	24.3838	- 0.838	28.5417	-1.1602

b) Correlation between QI scores for fresh skinless fillets and storage time in ice.

Var. X & Var. Y	Correlations Marked correlations are significant at $p < 0.05$ (Casewise deletion of missing data)										
	Mean	Std. Dv.	r(X, Y)	r^2	t	p	N	Constant dep: Y	Slope dep: Y	Constant dep: X	Slope dep: X
Days in ice	9.3333	5.8309									
QI score fillet	18.2067	3.9260	-0.991	0.981	-19.042	0.0000*	9	24.4312	-0.667	36.116	-1.471

c) Correlation between QI scores for cooked fillets and storage time in ice.

Var. X & Var. Y	Correlations Marked correlations are significant at $p < 0.05$ (Casewise deletion of missing data)										
	Mean	Std. Dv.	r(X, Y)	r^2	t	p	N	Constant dep: Y	Slope dep: Y	Constant dep: X	Slope dep: X
Days in ice	9.3333	5.8309									
QI score H&G	17.5183	7.7519	-0.934	0.873	-6.939	0.0002*	9	29.112	-1.242	21.6458	-0.7028

N.B. * denotes that it is statistically significant at $p < 0.05$.

Table 13. Results of two-way analysis of variance (ANOVA) to test if a significant statistical difference between quality scores for each quality attribute/parameter assessed (of fresh H&G and skinless fillets as well as cooked fillets) existed with storage time in ice (0°C).

a) Two-way ANOVA between quality scores for each quality attribute of fresh H&G assessed with storage time in ice.

Effect	Univariate Tests of significance for Quality score Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	3808.266	1	3808.266	6364.844	0.0000*
Days in ice	315.465	8	39.433	65.905	0.0000*
Quality parameter	58.714	3	19.571	32.710	0.0000*
Days in ice*Quality parameter	70.318	24	2.930	4.897	0.0000*
Error	112.486	188	0.598		

b) Two-way ANOVA between quality scores for each quality attribute of fresh skinless fillets assessed with storage time in ice.

Effect	Univariate Tests of significance for Quality score Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	4617.591	1	4617.591	6829.896	0.0000*
Days in ice	199.491	8	24.936	36.883	0.0000*
Quality parameter	30.249	3	10.083	14.914	0.0000*
Days in ice*Quality parameter	97.367	24	4.057	6.001	0.0000*
Error	129.808	192	0.676		

c) Two-way ANOVA between quality scores for each quality attribute of cooked fillets assessed with storage time in ice.

Effect	Univariate Tests of significance for Quality score Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	3184.912	1	3184.912	7768.172	0.0000*
Days in ice	359.679	8	44.960	109.660	0.0000*
Quality parameter	73.991	5	14.798	36.094	0.0000*
Days in ice*Quality parameter	201.049	40	5.026	12.259	0.0000*
Error	118.079	288	0.410		

N.B. * denotes that it is statistically significant at $p < 0.05$.

Appendix C: Results of ranked mean data

Quality parameter: Discolouration

Slide No.	Respondent																															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**Mean**	**SEM**
15 V 1	4	2	6	6	6	1	2	1	1	2	4	1	2	4	6	4	6	1	1	3	2	1	3	2	4	1	6	1	1	1	**2.833333**	**0.352604**
18 V 1	4	4	6	4	3	4	3	1	1	4	4	3	3	6	1	6	6	1	6	3	2	1	3	4	5	1	6	1	1	4	**3.366667**	**0.330389**
6 V 1	5	3	4	3	6	1	3	1	1	3	4	1	3	6	6	4	6	1	1	4	6	3	3	4	6	3	6	3	4	4	**3.6**	**0.316591**
10 V 1	4	6	6	3	6	3	3	1	4	4	4	3	2	4	2	5	4	3	1	3	3	3	4	4	5	4	6	1	4	3	**3.6**	**0.256412**
8 V 2	4	6	6	3	4	3	3	4	6	2								6	6	3	3	1	3	4	4	3	4	1	4	4	**3.782609**	**0.307717**
1 V 2	5	4	6	6	6	3	4	1	6	3								1	4	4	3	3	4	2	6	3	3	4	4	4	**3.869565**	**0.309943**
12 V 3	4	6	6	4	4	4	3	3	6		5	3	3	5	3	4	4	3	4	4	3	3	4	4	4	3	4	2	4	4	**3.896552**	**0.181298**
16 V 2	4	4	6	4	6	6	4	3	4	3								6	3	3	3	4	4	4	6	4	3	1	4	1	**3.913043**	**0.294301**
3 V 2	5	6	4	4	4	6	3	1	6	4								3	4	4	6	2	4	4	6	1	6	3	3	4	**4.043478**	**0.31734**
19 V 3	4	6	4	4	4	4	3	4	6		4	4	3	6	3	4	5	4	4	4	4	3	4	6	5	3	6	4	4	4	**4.241379**	**0.176551**
14 V 2	5	6	4	3	3	4	5	4	6	4								4	4	4	3	1	4	6	6	3	6	6	3	4	**4.26087**	**0.275605**
10 V 2	5	6	6	4	6	4	3	3	4	6								3	4	6	6	2	3	6	4	3	4	3	4	4	**4.304348**	**0.269935**
17 V 1	6	6	6	6	6	3	4	1	3	6	6	5	6	6	6	4	6	1	1	4	6	4	4	4	6	4	6	1	3	3	**4.433333**	**0.324067**
13 V 2	5	6	6	4	4	4	5	4	6	5								5	4	4	4	2	4	6	4	4	3	4	4	6	**4.478261**	**0.216599**
16 V 1	5	6	4	6	6	4	5	4	4	6	6	4	4	6	6	3	6	3	1	6	4	4	4	6	6	3	4	3	4	4	**4.566667**	**0.238209**
8 V 1	6	6	3	6	6	1	5	4	1	6	6	6	6	6	6	5	6	1	1	6	3	3	6	4	6	4	6	1	6	6	**4.6**	**0.351025**
2 V 2	5	6	6	4	6	4	3	4	4	6								6	4	4	4	4	4	4	6	4	6	4	4	4	**4.608696**	**0.206027**
5 V 1	5	5	4	4	6	4	5	6	1	5	6	5	4	6	6	5	6	4	4	4	3	4	4	6	6	4	6	3	3	6	**4.666667**	**0.226586**
7 V 1	6	6	3	6	6	6	4	1	6	6	6	6	4	6	6	3	6	1	1	4	6	4	6	4	6	4	6	1	6	4	**4.666667**	**0.326364**
14 V 3	5	6	6	4	4	4	4	6	6		6	3	5	6	4	4	6	5	1	4	6	4	6	4	6	3	4	3	6	6	**4.724138**	**0.242608**
11 V 2	5	6	6	6	6	4	5	3	6	4								6	4	6	6	2	6	6	6	3	3	3	4	4	**4.782609**	**0.280855**
12 V 1	5	6	6	4	6	4	5	3	6	5	6	5	4	4	6	6	6	5	3	4	4	4	4	6	6	4	6	4	3	4	**4.8**	**0.194168**
9 V 1	5	6	4	6	6	3	4	6	6	3	6	6	4	6	6	5	6	1	6	3	6	4	4	6	6	3	6	4	6	4	**4.9**	**0.250746**
14 V 1	6	6	6	4	6	4	5	4	4	6	6	5	6	6	6	3	6	5	1	4	4	4	6	6	6	4	6	6	4	6	**5.033333**	**0.227345**
6 V 3	6	6	6	6	6	3	5	3	6		6	5	6	6	6	4	6	1	1	6	6	4	6	6	6	4	6	2	6	6	**5.034483**	**0.295628**
13 V 3	5	6	6	6	4	4	4	6	6		6	6	4	6	6	4	6	3	1	4	6	4	6	6	6	3	6	6	4	6	**5.034483**	**0.245565**
15 V 3	6	6	4	3	6	4	5	4	6		6	5	6	6	6	4	6	3	1	6	6	4	6	6	6	4	4	6	6	6	**5.068966**	**0.242783**
1 V 3	5	6	6	6	6	4	4	6	6		5	6	5	6	6	6	5	1	6	3	5	3	6	6	6	1	6	6	6	6	**5.137931**	**0.270577**

5 V 3	5	6	4	4	6	6	3	6	6		5	5	6	6	4	5	6	5	6	1	5	3	6	6	6	6	6	6	4	6	**5.137931**	**0.231317**
13 V 1	6	6	4	6	6	4	5	4	4	6	6	5	6	6	6	6	6	5	3	4	6	4	4	6	6	3	6	6	6	4	**5.166667**	**0.192284**
1 V 1	5	6	6	6	6	4	6	4	6	5	6	5	4	6	6	6	6	5	1	6	6	4	6	4	6	4	6	6	4	6	**5.233333**	**0.212718**
6 V 2	6	6	4	6	6	6	5	4	4	6								5	4	6	6	6	6	6	6	4	4	4	6	6	**5.304348**	**0.19311**
3 V 3	6	6	6	6	6	6	4	4	6		6	5	5	6	6	6	6	3	4	4	4	4	6	6	6	6	6	4	6	6	**5.344828**	**0.180829**
20 V 2	6	6	6	4	4	4	5	6	6	6								3	6	6	6	4	6	6	6	6	6	6	4	6	**5.391304**	**0.206027**
7 V 3	6	6	6	6	6	6	5	4	6		6	5	6	6	6	6	6	3	4	6	6	4	6	6	6	6	3	3	6	6	**5.413793**	**0.195499**
8 V 3	5	6	6	6	6	6	5	6	6		5	5	5	6	4	6	6	5	6	3	5	4	6	6	6	6	6	4	6	6	**5.448276**	**0.15366**
16 V 3	6	6	6	6	6	6	6	4	4		6	6	6	6	6	5	6	6	1	6	6	4	6	6	6	4	6	4	6	6	**5.448276**	**0.21396**
4 V 2	6	6	6	6	6	6	5	4	6	6								3	4	4	6	6	6	6	6	6	6	4	6	6	**5.478261**	**0.19751**
15 V 2	6	6	6	6	6	1	6	6	6	6								5	6	4	6	4	6	6	6	6	6	4	6	6	**5.478261**	**0.250451**
20 V 3	6	6	6	6	6	6	6	6	6		6	4	6	6	6	5	6	4	4	4	6	4	6	6	6	4	4	6	6	6	**5.482759**	**0.161738**
11 V 1	6	6	6	6	6	4	6	4	6	6	6	5	6	6	6	5	6	5	4	3	5	4	6	6	6	6	6	6	6	6	**5.5**	**0.157203**
19 V 1	5	6	6	6	6	4	4	4	6	6	6	5	6	6	6	6	6	5	6	4	6	4	6	6	6	4	6	6	6	6	**5.5**	**0.149712**
10 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	5	1	4	4	4	6	6	6	6	6	6	4	6	**5.517241**	**0.208328**
17 V 3	6	6	6	6	6	6	5	6	6		6	5	6	6	6	5	6	5	4	4	6	4	6	6	6	4	4	6	6	6	**5.517241**	**0.145716**
5 V 2	6	6	6	6	6	4	6	6	6	6								3	4	6	6	6	6	6	6	6	6	6	4	6	**5.608696**	**0.185855**
2 V 3	6	6	6	6	6	6	6	4	6		6	5	6	6	6	6	6	5	4	6	6	6	6	6	6	6	3	4	6	6	**5.62069**	**0.152272**
4 V 1	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	4	6	6	6	4	6	4	6	6	**5.633333**	**0.139649**
9 V 2	6	6	6	6	6	6	5	6	6	6								6	6	4	5	4	6	6	6	6	6	6	4	6	**5.652174**	**0.148892**
18 V 2	6	6	6	6	6	6	6	6	6	6								3	3	6	4	6	6	6	6	6	6	6	6	6	**5.652174**	**0.194882**
11 V 3	6	6	6	6	6	6	6	6	4		6	3	6	6	6	6	6	6	1	6	6	6	6	6	6	6	6	6	6	6	**5.655172**	**0.20628**
4 V 3	6	6	6	6	6	4	5	6	6		6	5	6	6	6	6	6	5	6	4	4	6	6	6	6	6	6	6	6	6	**5.689655**	**0.12261**
19 V 2	6	6	6	6	6	6	6	6	4	6								6	6	6	6	1	6	6	6	6	6	6	6	6	**5.695652**	**0.230439**
2 V 1	6	6	6	6	6	6	5	6	6	6	5	6	6	6	6	6	5	6	6	4	6	4	6	6	6	6	6	4	6	6	**5.7**	**0.118903**
9 V 3	6	6	6	6	6	6	5	6	6		6	5	6	6	6	6	6	5	6	6	5	6	6	6	6	6	6	6	6	6	**5.862069**	**0.065166**
12 V 2	6	6	6	6	6	6	6	6	6	6								5	6	6	6	6	6	6	6	6	4	6	6	6	**5.869565**	**0.095436**
3 V 1	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	**5.933333**	**0.046321**
20 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	**5.933333**	**0.066667**
17 V 2	6	6	6	6	6	6	6	6	6	6								5	6	6	6	6	6	6	6	6	6	6	6	6	**5.956522**	**0.043478**
18 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	**5.965517**	**0.034483**
7 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**

Quality parameter: Blood on the fatline

Slide No.	Respondent																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean	SEM
15 V 1	4	6	3	3	6	1	3	1	1	6	6	3	3	6	1	5	6	6	6	1	6	1	1	2	3	1	6	1	1	1	3.333333	0.396343
6 V 1	4	6	6	3	6	1	6	1	1	6	3	2	6	4	1	4	4	6	1	3	2	1	3	4	1	3	6	3	1	4	3.4	0.351025
1 V 2	5	6	4	4	6	4	6	1	3	6								3	2	6	6	3	1	2	4	1	6	3	3	4	3.869565	0.368226
10 V 2	5	4	3	6	4	6	2	3	4	6								6	4	4	6	1	3	4	1	3	4	3	3	4	3.869565	0.309943
3 V 2	5	4	6	6	6	4	3	1	4	6								6	6	4	4	2	1	4	4	4	6	1	3	4	4.086957	0.349551
19 V 2	3	6	6	6	6	6	2	3	4	6								6	1	6	4	4	3	4	1	3	6	1	4	3	4.086957	0.376761
20 V 2	4	4	4	6	6	6	3	6	4	6								6	1	4	5	2	1	4	4	3	6	6	4	4	4.304348	0.329299
17 V 1	4	6	6	6	6	3	2	1	1	6	6	3	6	6	6	4	6	6	6	6	6	3	1	2	5	3	5	3	4	4	4.4	0.327302
8 V 2	5	4	4	6	6	6	3	3	4	6								6	3	4	6	6	3	4	4	3	6	3	3	4	4.434783	0.265441
16 V 1	4	6	6	6	6	4	2	4	1	6	6	6	4	6	6	3	6	4	6	6	6	1	4	4	5	3	4	4	3	4	4.533333	0.282572
18 V 1	4	6	6	6	6	6	3	1	1	6	6	6	4	6	6	6	6	6	6	6	6	3	3	2	4	1	6	1	3	4	4.533333	0.344858
7 V 1	3	6	6	6	6	1	6	1	1	6	6	6	6	6	6	5	6	1	6	6	1	6	4	6	5	4	6	1	3	6	4.6	0.370151
11 V 2	5	6	6	6	6	3	5	3	1	6								6	6	6	6	2	3	4	6	3	6	6	1	6	4.695652	0.368925
14 V 2	4	4	6	6	6	6	6	4	4	6								6	4	6	6	2	4	4	4	4	6	4	3	4	4.73913	0.25318
18 V 2	4	6	4	6	6	6	3	4	6	6								6	1	6	6	4	4	4	4	3	6	6	4	4	4.73913	0.289591
5 V 2	5	6	6	6	6	4	5	4	3	6								6	4	4	4	6	4	4	4	4	3	6	4	6	4.782609	0.2263
16 V 2	4	4	6	6	6	6	3	4	4	6								6	6	6	6	4	4	4	6	4	6	4	3	4	4.869565	0.237779
6 V 2	5	6	6	6	6	6	5	4	1	6								6	6	4	6	4	3	6	6	4	4	4	4	6	4.956522	0.277469
9 V 1	4	6	6	6	6	6	6	1	4	6	6	4	6	6	3	5	6	2	6	6	6	3	6	6	6	4	6	4	4	4	5	0.258199
12 V 3	4	6	6	6	6	6	4	6	6		6	6	3	6	6	5	6	6	3	6	6	4	3	4	6	4	6	2	4	4	5.034483	0.235322
13 V 2	5	6	6	6	6	6	4	4	4	6								5	4	6	6	2	4	6	6	4	6	6	4	4	5.043478	0.23922
8 V 1	6	6	6	6	6	6	6	4	1	5	6	6	5	6	3	5	6	6	6	3	6	1	6	6	5	6	6	1	6	6	5.1	0.296919
4 V 3	5	6	4	6	6	6	4	6	6		6	6	5	6	6	6	6	4	4	6	6	3	4	4	4	4	6	4	6	6	5.206897	0.188194
2 V 2	5	6	6	6	6	6	4	4	4	6								6	6	6	6	4	4	4	4	4	6	6	6	6	5.26087	0.20096
10 V 3	5	6	6	6	6	6	2	4	6		6	6	6	6	6	6	6	5	1	6	6	4	4	4	6	4	6	6	6	6	5.275862	0.242608
6 V 3	5	6	6	6	6	3	4	6	6		6	6	5	6	6	6	6	5	4	6	6	6	4	4	6	4	6	2	6	6	5.310345	0.205248
16 V 3	5	6	6	6	6	6	3	6	4		6	6	4	6	6	5	6	6	6	6	6	4	3	6	6	4	6	4	6	4	5.310345	0.192875
19 V 3	5	6	6	6	6	6	4	4	6		6	6	5	6	6	5	6	6	4	6	6	4	4	6	6	4	6	4	4	6	5.344828	0.166653
14 V 3	6	6	6	6	6	6	3	6	6		6	6	6	6	6	5	6	5	1	6	4	4	4	6	6	4	6	6	6	6	5.37931	0.224233
19 V 1	5	6	6	6	6	6	4	4	4	6	6	6	6	6	6	6	6	5	6	6	6	4	4	4	6	4	6	6	6	4	5.4	0.163299
5 V 3	5	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	5	4	6	6	3	4	4	4	4	6	6	4	6	5.413793	0.175587
10 V 1	5	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	5	4	6	1	6	4	5.433333	0.207244

3 V 3	5	6	4	6	6	6	5	6	4		6	6	6	6	6	6	6	6	4	6	6	4	4	6	6	4	6	4	6	6	**5.448276**	**0.161476**
9 V 3	6	6	6	6	6	6	3	4	6		6	6	6	6	6	6	6	5	6	4	6	4	4	6	6	4	6	4	6	6	**5.448276**	**0.17607**
1 V 1	5	6	4	6	6	6	6	4	6	6	6	6	6	6	6	6	6	5	6	6	2	4	6	6	6	4	6	4	6	6	**5.466667**	**0.184037**
12 V 1	4	6	6	6	6	6	6	4	4	6	6	6	6	6	6	6	6	5	6	6	6	3	4	6	6	4	6	6	6	4	**5.466667**	**0.171091**
20 V 3	6	6	6	6	6	6	4	6	6		6	6	6	6	6	5	6	6	6	4	6	4	4	6	6	6	4	4	4	6	**5.482759**	**0.161738**
14 V 1	5	6	6	6	6	6	6	3	4	6	6	6	6	6	3	2	6	6	6	6	6	4	6	6	6	6	6	6	6	6	**5.5**	**0.202001**
15 V 3	6	6	6	6	6	6	4	4	6		6	6	5	6	6	5	6	6	6	6	6	4	4	6	4	4	6	6	6	6	**5.517241**	**0.153936**
13 V 1	5	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	5	6	6	6	4	6	4	6	4	4	4	6	6	**5.533333**	**0.149584**
13 V 3	5	6	6	6	6	6	4	6	6		6	6	6	6	6	5	6	6	6	6	4	4	3	6	6	4	6	6	6	6	**5.551724**	**0.161476**
5 V 1	6	6	6	6	6	6	6	6	1	6	6	6	6	6	6	5	6	6	6	6	6	3	6	6	4	6	6	4	6	6	**5.566667**	**0.207244**
7 V 3	5	6	6	6	6	3	5	4	6		6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	6	6	6	**5.62069**	**0.152272**
4 V 2	6	6	6	6	6	4	6	4	4	6								6	6	6	6	6	6	4	6	6	6	6	6	6	**5.652174**	**0.161621**
1 V 3	4	6	6	6	6	6	5	6	6		6	6	6	6	6	6	6	6	6	6	6	3	6	6	6	4	6	4	6	6	**5.655172**	**0.151152**
11 V 1	5	6	6	6	6	6	6	3	4	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	4	6	6	**5.666667**	**0.146478**
8 V 3	6	6	6	6	6	6	4	6	6		6	6	6	6	6	6	6	5	6	6	6	4	6	6	4	4	6	6	6	6	**5.689655**	**0.132273**
11 V 3	6	6	6	6	6	6	5	6	6		6	3	6	6	6	6	6	6	1	6	6	6	6	6	6	6	6	6	6	6	**5.689655**	**0.199157**
17 V 3	5	6	6	6	6	6	4	6	6		6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	4	4	6	6	6	**5.689655**	**0.132273**
2 V 1	6	6	6	6	6	6	6	4	6	6	6	6	3	6	6	6	6	6	6	6	6	4	6	6	6	6	6	4	6	6	**5.7**	**0.145033**
9 V 2	5	6	6	6	6	6	5	6	4	6								6	6	6	6	4	6	6	6	6	6	6	6	6	**5.73913**	**0.129111**
17 V 2	5	6	6	6	6	6	5	6	6	6								6	6	6	6	4	4	6	6	6	6	6	6	6	**5.73913**	**0.129111**
2 V 3	6	6	6	6	6	6	5	6	4		6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	4	6	6	**5.758621**	**0.118021**
3 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	1	6	**5.766667**	**0.177358**
4 V 1	6	6	6	6	6	3	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	**5.833333**	**0.118257**
12 V 2	6	6	6	6	6	6	5	6	6	6								6	6	6	6	6	6	6	6	6	4	6	6	6	**5.869565**	**0.095436**
15 V 2	6	6	6	6	6	6	5	6	6	6								6	6	6	6	4	6	6	6	6	6	6	6	6	**5.869565**	**0.095436**
20 V 1	6	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.966667**	**0.033333**
7 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
18 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**

Quality parameter: Blood clots

Slide No.	Respondent 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean	SEM
8 V 1	4	6	6	6	6	1	3	1	1	1	2	1	3	3	3	4	3	1	3	1	6	1	1	1	1	4	6	1	1	1	2.733333	0.358584
7 V 1	3	6	6	6	6	6	3	1	4	1	6	1	6	6	1	3	6	6	1	1	6	1	1	2	1	1	6	1	1	1	3.333333	0.427054
6 V 3	4	6	6	6	6	3	3	6	4		3	3	2	4	2	4	6	6	1	1	3	1	3	2	3	3	6	2	1	3	3.551724	0.327651
15 V 1	4	5	6	6	6	1	3	1	1	1	6	1	6	6	6	4	4	6	6	6	6	1	3	2	3	4	4	1	1	1	3.7	0.387001
15 V 3	4	6	6	6	4	3	4	3	1		4	4	5	5	4	5	4	3	1	4	6	1	1	4	3	4	4	4	1	4	3.724138	0.284651
16 V 1	5	5	6	3	4	4	4	1	3	4	4	3	4	4	6	3	3	6	1	4	6	1	3	4	4	3	4	4	3	4	3.766667	0.242986
1 V 2	4	6	6	6	3	6	4	1	1	3								6	3	3	6	1	3	2	4	3	4	3	6	4	3.826087	0.359249
6 V 2	4	6	6	3	3	4	4	3	4	4								6	4	4	3	1	1	4	4	4	3	6	3	4	3.826087	0.278705
17 V 1	4	4	6	4	4	6	3	1	6	4	4	3	3	4	3	4	3	6	1	4	3	1	1	4	3	6	6	4	6	4	3.833333	0.283992
9 V 1	4	3	6	6	6	1	4	1	1	3	5	6	4	6	3	6	6	6	6	3	4	1	1	4	4	4	6	3	3	1	3.9	0.340216
4 V 2	5	6	6	4	4	4	4	1	1	4								6	3	4	6	1	3	4	4	4	3	6	3	4	3.913043	0.320036
3 V 1	5	3	4	4	4	4	5	1	1	5	5	6	4	5	3	5	4	6	1	4	4	1	4	2	2	4	6	6	6	4	3.933333	0.287411
6 V 1	6	3	6	6	6	1	4	1	1	3	6	6	6	6	6	4	4	6	1	2	6	1	4	4	5	4	6	3	1	1	3.966667	0.369788
2 V 3	5	6	3	4	4	4	4	3	4		4	3	4	4	4	5	5	6	6	3	4	1	4	4	4	4	3	4	3	4	4	0.192213
7 V 3	5	6	4	4	3	4	5	3	4		5	3	4	5	3	6	6	6	4	4	2	1	4	4	4	4	3	3	3	4	4	0.221948
4 V 1	5	5	6	3	4	6	4	1	6	3	6	6	4	5	6	5	4	6	1	3	4	1	3	4	2	4	6	6	1	1	4.033333	0.326892
5 V 2	5	6	4	4	3	6	4	3	1	5								6	6	4	6	1	3	4	4	4	3	4	3	4	4.043478	0.298072
18 V 1	5	5	4	6	6	6	5	1	1	5	4	3	6	5	4	5	6	1	3	4	6	1	3	4	5	4	6	1	4	4	4.1	0.308314
13 **V 1**	5	3	6	4	2	4	4	3	4	5	5	6	4	6	3	6	4	5	3	3	4	1	4	4	4	4	6	4	4	4	4.133333	0.218318
20 V 3	5	6	4	4	4	4	5	3	1		5	5	5	5	4	5	6	6	4	4	5	1	4	4	4	4	6	4	1	4	4.206897	0.250021
11 V 2	4	6	6	3	6	6	3	3	1	6								6	6	4	4	1	1	4	6	4	6	6	1	4	4.217391	0.392401
16 V 2	5	4	6	6	3	6	5	4	4	3								6	6	4	6	1	3	4	6	4	6	4	4	3	4.478261	0.287208
16 V 3	5	6	6	3	4	3	6	6	4		6	6	6	6	6	6	6	6	1	6	3	1	1	6	3	4	4	6	1	4	4.517241	0.338865
1 V 1	5	6	6	4	4	1	5	3	4	5	4	6	6	5	4	5	4	5	3	4	6	1	6	4	6	4	6	4	6	6	4.6	0.251889
19 V 3	5	6	6	6	6	6	5	3	4		5	4	5	5	4	4	5	6	4	6	6	1	4	4	4	4	4	4	4	4	4.62069	0.212965
12 V 1	5	6	4	4	6	6	4	4	4	5	6	5	5	6	5	5	6	6	3	4	6	1	3	6	4	4	6	6	1	4	4.666667	0.255214
5 V 1	5	5	6	6	6	6	4	3	6	5	4	6	6	4	6	4	3	6	6	4	6	1	4	4	4	4	6	4	4	4	4.733333	0.229609
11 V 1	5	6	6	4	6	6	4	4	4	5	5	5	5	6	5	5	6	6	4	4	6	1	4	4	5	4	6	4	3	4	4.733333	0.208626
19 V 1	5	5	6	6	4	6	5	3	4	5	4	6	5	5	6	6	6	6	3	4	5	3	4	4	5	4	6	4	4	4	4.766667	0.183725
3 V 2	6	6	6	6	6	6	6	1	1	6								6	6	6	6	6	1	6	4	4	4	1	4	6	4.782609	0.402347
17 V 3	5	6	4	6	6	4	5	6	4		5	6	5	5	4	5	6	6	4	4	4	1	4	6	4	4	4	6	4	6	4.793103	0.212766
14 V 1	5	5	6	6	4	6	5	3	4	5	5	6	5	5	6	1	4	6	6	4	6	1	4	6	5	4	6	6	3	6	4.8	0.255514

13 V 3	5	6	6	4	6	4	5	3	4		6	6	5	6	4	4	6	6	4	6	6	1	3	6	6	4	6	3	3	6	**4.827586**	**0.253395**
10 V 2	6	4	6	6	6	6	6	3	4	5								6	6	6	3	6	6	6	4	4	4	3	4	6	**5.043478**	**0.247344**
14 V 2	5	4	6	6	6	6	5	4	4	6								6	4	4	6	2	6	6	4	4	6	6	4	6	**5.043478**	**0.23922**
14 V 3	5	6	6	6	6	6	5	6	6		6	4	6	6	6	6	6	6	1	6	4	1	4	6	6	4	4	4	3	6	**5.068966**	**0.271518**
13 V 2	5	4	6	6	6	6	5	4	4	6								6	6	4	6	6	4	6	6	6	4	4	4	4	**5.130435**	**0.201813**
2 V 2	5	6	6	6	6	6	5	4	4	6								6	6	6	6	1	6	4	6	4	6	6	4	6	**5.26087**	**0.26087**
8 V 2	6	4	6	6	6	6	6	4	4	6								6	6	6	6	6	6	6	3	6	1	4	6		**5.304348**	**0.277159**
12 V 3	6	6	6	6	6	6	6	6	6		6	4	6	6	4	6	6	6	6	6	6	1	6	6	6	4	4	2	6	6	**5.413793**	**0.240675**
10 V 1	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	5	6	6	6	4	6	2	6	6	5	6	6	1	4	6	**5.433333**	**0.228354**
18 V 3	6	6	6	6	6	4	5	6	4		6	6	6	6	5	6	6	6	4	4	6	4	6	6	6	6	6	6	6	6	**5.586207**	**0.144839**
4 V 3	5	6	6	6	4	6	5	6	6		6	6	6	6	5	6	6	6	6	6	6	3	6	6	6	6	6	4	6	6	**5.655172**	**0.142772**
11 V 3	6	6	6	6	6	6	6	6	1		6	6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	6	6	6	6	**5.655172**	**0.239436**
2 V 1	6	5	6	6	6	6	6	6	6	6	6	6	6	6	3	6	6	6	4	6	6	4	6	6	6	6	6	4	6	6	**5.666667**	**0.146478**
9 V 2	6	4	6	6	6	6	6	6	4	5								6	6	6	6	6	6	6	6	6	6	6	6	6	**5.782609**	**0.125054**
1 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	6	4	6	**5.793103**	**0.115107**
19 V 2	6	4	6	6	6	6	6	6	4	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**5.826087**	**0.120148**
20 V 2	6	4	6	6	6	6	6	6	4	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**5.826087**	**0.120148**
3 V 3	6	6	6	6	6	6	6	3	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	**5.827586**	**0.122263**
10 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	6	6	6	6	**5.827586**	**0.172414**
5 V 3	5	6	6	6	6	6	4	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.896552**	**0.075996**
12 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	4	6	6	6	**5.913043**	**0.086957**
8 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	**5.931034**	**0.068966**
9 V 3	6	6	6	6	6	6	6	6	4		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.931034**	**0.068966**
20 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
7 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
15 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
17 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
18 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**

Quality parameter: Gaping

Slide No.	Respondent 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean	SEM
15 V 1	3	6	6	3	6	6	6	1	1	6	4	6	6	6	1	6	3	1	6	1	6	1	1	2	4	1	4	1	6	1	**3.7**	**0.410074**
3 V 3	5	6	4	6	6	6	6	6	6		4	6	6	6	6	5	3	3	6	3	6	3	6	2	4	4	6	4	6	6	**5.034483**	**0.240498**
4 V 2	6	6	6	6	6	6	6	1	4	6								6	6	3	4	6	3	4	4	6	6	6	6	3	**5.043478**	**0.30463**
16 V 2	6	4	6	6	6	6	6	6	4	6								6	6	6	6	6	3	4	6	4	4	4	4	3	**5.130435**	**0.237779**
17 V 2	6	6	6	6	6	6	6	1	6	6								6	6	3	3	6	4	4	4	6	4	6	6	6	**5.173913**	**0.292544**
13 V 2	6	4	6	6	6	6	6	4	4	6								6	6	4	6	6	4	4	6	3	6	3	6	6	**5.217391**	**0.23487**
4 V 1	6	6	6	6	6	6	6	6	1	6	6	1	6	6	6	5	6	6	6	3	6	6	4	2	4	6	6	6	6	6	**5.266667**	**0.279299**
8 V 2	6	4	4	6	6	6	6	6	4	6								6	6	6	6	6	6	4	6	3	6	1	6	6	**5.304348**	**0.277159**
18 V 1	6	5	6	6	6	6	6	4	1	6	6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	4	6	1	6	6	**5.366667**	**0.251356**
19 V 2	6	4	6	6	6	6	6	6	4	6								6	6	4	4	6	6	6	4	6	4	6	6	6	**5.478261**	**0.187237**
11 V 2	6	6	6	6	6	6	6	4	6	6								6	6	4	6	6	6	4	6	3	4	6	6	6	**5.521739**	**0.19751**
12 V 2	6	6	4	6	6	6	6	6	6	6								6	6	4	5	2	6	6	6	6	4	6	6	6	**5.521739**	**0.216599**
15 V 2	6	6	4	6	6	6	6	6	6	6								6	6	4	5	6	4	4	4	6	6	6	6	6	**5.521739**	**0.176366**
7 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	1	6	1	**5.533333**	**0.243222**
14 V 2	6	6	6	6	6	6	6	4	4	6								6	6	6	6	6	6	4	6	4	6	4	6	6	**5.565217**	**0.175878**
16 V 1	6	6	6	6	6	6	6	6	1	6	6	6	6	6	6	4	6	6	6	6	6	6	6	4	6	4	6	4	6	6	**5.566667**	**0.201622**
13 V 1	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	4	6	3	6	6	6	4	4	6	6	6	**5.633333**	**0.155241**
17 V 1	6	6	4	6	6	6	6	6	6	6	4	6	6	6	6	5	6	6	6	6	6	6	6	4	6	4	6	4	6	6	**5.633333**	**0.139649**
6 V 2	6	6	6	6	6	6	6	6	4	6								6	6	4	6	6	6	6	6	4	6	6	6	4	**5.652174**	**0.161621**
10 V 2	6	6	3	6	6	6	6	6	4	6								6	6	6	6	6	6	6	6	3	6	6	6	6	**5.652174**	**0.194882**
8 V 1	6	6	6	6	6	6	6	6	1	6	6	6	6	6	6	5	6	6	6	6	6	6	6	4	6	4	6	6	6	6	**5.666667**	**0.187747**
14 V 1	5	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	4	6	1	6	6	6	6	6	6	6	6	**5.666667**	**0.187747**
3 V 2	5	6	6	4	6	6	6	6	6	6								6	6	6	6	6	6	4	6	4	6	6	6	6	**5.695652**	**0.146565**
7 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	4	6	6	6	6	4	4	6	4	6	6	**5.724138**	**0.130333**
12 V 3	6	6	6	6	6	6	6	3	6		5	6	6	6	6	6	6	6	6	4	6	6	6	6	6	4	6	6	6	6	**5.724138**	**0.139462**
1 V 1	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	4	6	6	6	5	5	4	6	6	6	6	**5.733333**	**0.11679**
6 V 1	6	6	6	6	6	6	6	6	4	6	3	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	3	6	6	**5.733333**	**0.151113**
9 V 1	6	6	6	6	6	6	6	4	4	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	6	6	6	**5.733333**	**0.126249**
18 V 2	6	6	6	6	6	6	6	4	6	6								6	6	6	6	4	6	6	4	6	6	6	6	6	**5.73913**	**0.143604**
4 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	5	6	6	6	6	6	6	6	2	6	6	6	4	6	6	**5.758621**	**0.154212**
20 V 3	5	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	4	4	4	6	6	6	6	**5.758621**	**0.118021**
10 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	1	6	6	**5.766667**	**0.177358**

7 V 2	6	6	6	6	6	6	6	6	6	6								6	6	4	5	6	6	6	4	6	6	6	6	6	5.782609	0.125054
19 V 1	6	6	6	6	6	6	6	4	4	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.8	0.111417
1 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	4	6	4	6	6	6	6	5.826087	0.120148
2 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	4	6	4	6	6	6	6	5.826087	0.120148
5 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	4	6	6	6	4	5.826087	0.120148
3 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	3	6	6	6	6	6	4	6	6	6	6	5.833333	0.118257
1 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	6	5.862069	0.095774
2 V 3	6	6	6	6	6	6	6	4	4		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5.862069	0.095774
6 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	6	6	6	6	6	5.862069	0.095774
10 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	6	5.862069	0.095774
13 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	6	5.862069	0.095774
14 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	4	6	6	5.862069	0.095774
16 V 3	6	6	4	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.862069	0.095774
5 V 3	6	6	6	6	6	6	5	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.896552	0.075996
9 V 3	6	6	6	6	6	6	6	6	6		6	6	5	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	5.896552	0.075996
2 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	5	6	6	6	6	6	6	6	6	6	5.9	0.0735
20 V 2	6	4	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	5.913043	0.086957
11 V 3	6	6	6	6	6	6	6	6	4		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5.931034	0.068966
15 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	5.931034	0.068966
17 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.931034	0.068966
19 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.931034	0.068966
11 V 1	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5.933333	0.066667
12 V 1	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5.933333	0.066667
5 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0
20 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0
9 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	6	0
8 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0
18 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0

Quality parameter: Occurrence of parasites

Slide No.	Respondent Number																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Mean	SEM
7 V 1	6	6	6	6	6	6	6	6	6	6	2	1	2	2	1	6	2	6	1	6	6	6	4	2	6	6	6	6	6	6	4.766667	0.36098
11 V 2	6	6	6	6	6	6	6	1	6	3								6	1	6	6	6	4	4	1	4	6	6	6	6	4.956522	0.374473
8 V 2	6	6	6	6	6	6	6	1	6	6								6	6	1	6	6	1	4	6	3	6	6	6	6	5.130435	0.373554
16 V 3	6	6	6	6	6	6	6	3	6		4	3	4	6	4	5	3	6	6	6	6	6	6	2	3	4	6	6	6	6	5.137931	0.241731
15 V 1	3	6	6	6	6	6	6	1	1	6	6	6	6	6	4	6	6	6	6	3	6	6	6	2	6	4	6	6	6	6	5.2	0.289272
18 V 1	6	6	6	6	6	6	6	4	1	6	6	6	6	6	6	6	6	1	6	6	6	6	1	4	6	1	6	6	6	6	5.2	0.319482
16 V 2	6	4	6	6	6	6	6	1	4	6								6	6	1	6	6	6	6	6	6	6	6	4	6	5.304348	0.317069
8 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	2	6	5	6	6	6	6	6	6	4	4	6	3	6	6	6	6	5.433333	0.238209
6 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	3	6	6	6	6	6	1	3	6	6	6	6	6	6	6	5.466667	0.261443
14 V 3	6	6	6	6	6	6	6	3	6		5	6	6	5	4	6	5	6	6	6	6	6	6	6	3	4	4	6	6	6	5.482759	0.176311
9 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	2	6	6	2	6	6	6	6	6	4	6	6	6	6	6	6	6	5.5	0.24798
13 V 3	6	6	6	6	6	6	6	6	6		5	6	6	5	6	6	3	6	6	6	6	6	6	6	3	3	3	6	6	6	5.517241	0.196149
12 V 1	6	6	6	6	6	6	6	1	6	6	4	6	6	4	6	4	3	6	6	6	6	6	6	6	6	6	6	6	6	6	5.533333	0.212988
20 V 2	6	6	6	6	6	6	6	1	6	6								6	1	6	6	6	6	6	6	6	6	6	6	6	5.565217	0.300369
19 V 1	6	6	6	6	6	6	6	4	6	6	6	3	6	6	6	6	4	6	6	4	6	6	6	6	6	4	6	6	6	6	5.633333	0.155241
1 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	1	6	6	6	6	5.655172	0.239436
15 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	5	6	6	1	6	6	6	6	2	6	6	6	6	6	6	5.655172	0.217893
11 V 1	6	3	6	6	6	6	6	4	6	6	6	6	6	3	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6	6	5.666667	0.161411
16 V 1	6	6	6	6	6	6	6	3	6	6	6	6	6	6	6	4	6	6	6	1	6	6	6	6	6	6	6	6	6	6	5.666667	0.199616
19 V 2	6	4	6	6	6	6	6	6	6	6								6	6	1	6	6	6	6	6	6	6	6	6	6	5.695652	0.230439
4 V 1	6	6	6	6	6	6	6	6	6	6	4	5	6	6	2	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	5.7	0.160101
17 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	4	6	6	6	6	5.7	0.186622
10 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	3	6	6	6	6	3	4	6	6	6	6	5.724138	0.156127
20 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	4	6	6	6	6	6	4	6	4	4	6	6	6	6	5.724138	0.130333
5 V 2	6	6	6	6	6	6	6	4	6	6								6	6	4	6	6	6	6	4	6	6	6	6	6	5.73913	0.143604
8 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	1	6	6	6	6	5.758621	0.183394
13 V 1	6	6	6	6	6	6	6	3	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	4	6	6	6	6	6	6	5.766667	0.132902
14 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	5.766667	0.177358
1 V 2	6	6	6	6	6	6	6	1	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	5.782609	0.217391
4 V 2	6	6	6	6	6	6	6	1	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	5.782609	0.217391
20 V 1	6	6	6	6	6	6	6	1	6	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5.8	0.168836
12 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	2	6	6	6	6	6	6	6	6	5.826087	0.173913

13 V 2	6	4	6	6	6	6	6	4	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**5.826087**	**0.120148**
18 V 2	6	6	6	6	6	6	6	4	6	6								6	6	6	6	4	6	6	6	6	6	6	6	6	**5.826087**	**0.120148**
3 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	4	6	6	6	6	6	6	3	6	6	6	6	6	6	6	6	**5.827586**	**0.122263**
4 V 3	6	6	6	6	6	6	6	6	6		6	6	6	5	5	6	6	6	6	6	6	6	6	6	6	6	3	6	6	6	**5.827586**	**0.111737**
7 V 3	6	6	6	6	6	6	6	6	6		5	6	6	6	6	6	2	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.827586**	**0.140976**
6 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	2	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.862069**	**0.137931**
3 V 2	5	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	4	6	6	6	6	**5.869565**	**0.095436**
7 V 2	6	6	6	6	6	6	6	6	6	6								6	6	3	6	6	6	6	6	6	6	6	6	6	**5.869565**	**0.130435**
10 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	3	6	6	6	6	**5.869565**	**0.130435**
9 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	3	6	6	6	6	6	**5.896552**	**0.103448**
2 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	4	6	6	6	6	**5.913043**	**0.086957**
6 V 2	6	6	6	6	6	6	6	4	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**5.913043**	**0.086957**
11 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	**5.931034**	**0.068966**
17 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.931034**	**0.068966**
1 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	**5.933333**	**0.066667**
3 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	**5.933333**	**0.066667**
5 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.933333**	**0.046321**
5 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.965517**	**0.034483**
18 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	**5.965517**	**0.034483**
2 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
10 V 1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
9 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
14 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
15 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
17 V 2	6	6	6	6	6	6	6	6	6	6								6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
2 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
12 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**
19 V 3	6	6	6	6	6	6	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	**6**	**0**