Sveriges lantbruksuniversitet
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Faculty of Natural Resources and Agricultural Sciences

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– Establishment of a HACCP plan at VegaFish AB

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Abstract

The aquaculture industry is on a global scale becoming acutely aware of the demand for increased and intensified production systems to supply people with food fish. Moreover there are requirements for these systems to be more ecologically sound managements. As an attempt to address these concerns and after years of research, VegaFish AB has initiated an innovative project aiming to develop proprietary, highly cost efficient and environmentally sustainable systems for land-based shrimp production, based on the Biofloc Technology (BFT) system.

The European food law requires all food processing operators to provide food fit for human consumption which contributes to the public health and well-being. The food law is based on a preventive approach through implementation of prerequisite programs (PRP) and Hazard Analysis and Critical Control Point (HACCP) plans. VegaFish aims to meet not only the requirements of the international ISO 22000 standard addressing food safety but also the European Commission's intention to extend the food law regarding primary productions by the establishment of a HACCP plan.

Aquacultured products are in generally classified as low risk food in terms of incidence of food-borne disease outbreaks. Along with the fact that HACCP plans are not required, shrimp producers can seldom prove if any measures are taken to prevent food-borne disease outbreaks. Nevertheless, it is of great importance to understand the potential public health risks associated with a shrimp production to be able to manage them in an effective way. By the establishment of a HACCP plan and PRPs, VegaFish will work with a preventive approach on food-borne diseases.

The thesis aims to conduct a food safety hazard analysis and establish a HACCP plan for the shrimp production facilities in Uppsala and Bjuv and has been developed in collaboration with VegaFish. A number of control points controlled by the PRPs were determined along with one Critical Control Point (CCP) during the harvest regarding the need to keep the cold chain to prevent pathogen growth.

*Keywords:*HACCP, food safety, biofloc, shrimp, land-based aquaculture

Sammanfattning

Den globala vattenbruksindustrin pressas av ett påtagligt behov av en ökad och intensifierad produktion, samtidigt som kraven för miljömässig hållbarhet gör sig ständigt påminda. I ett försök att möta några av dessa utmaningar och efter år av forskning har företaget VegaFish AB inlett ett innovativt projekt som syftar till att starta ett egenutvecklat, kostnadseffektivt och miljömässigt hållbart system för landbaserad produktion av tropiska jätteräkor, baserat på den s.k. biofloc-metoden.

Europaunionens livsmedelslagstiftning kräver att varje livsmedelsföretag bidrar till att tillhandahålla konsumenter mat med hög livsmedelsstatus i strävan efter en god folkhälsa och högt välbefinnande. Livsmedelslagstiftningen ska förebygga risken för att hälsofarliga livsmedel hamnar på marknaden genom att livsmedelsföretagare definierar sanitära och hygieniska system dels genom grundförutsättningar (PRP), dels genom faroanalys och kritiska styrpunkter (HACCP). Genom implementering av HACCP-principerna syftar VegaFish till att tillgodose kraven för den internationella livsmedelssäkerhetsstandarden ISO 22000 men också den europeiska kommissionens avsikt att inom en överskådlig framtid utöka kraven för HACCP att även gälla primärproducenter, vilket inte är fallet idag.

Generellt klassas risken för att drabbas av livsmedelsburna sjukdomsutbrott från odlade fisk- och skaldjursprodukter som låg. Detta faktum i kombination med avsaknaden på legala krav att implementera HACCP-planer resulterar i att räkproducenter på ett globalt plan sällan kan visa vilka förebyggande åtgärder som vidtas inom en produktion för att i så stor utsträckning som möjligt undvika förekomsten av livmedelsburna sjukdomsutbrott. Icke desto mindre är det viktigt att noga förstå de potentiella riskerna associerade med tropiska jätteräkor för att kunna förebygga dem på ett effektivt sätt. Genom att etablera rutiner för PRPer och HACCP kan VegaFish på ett effektivt arbeta förebyggande.

Denna masteruppsats har syftat till att i samarbete med VegaFish genomföra en faroanalys och upprätta HACCP-plan för räkodlingsanläggningarna i Uppsala och Bjuv. Ett antal styrpunkter som kontrolleras av PRPerna har fastställts, tillsammans med en kritisk styrpunkt (CCP) under skörden i syfte att hålla kylkedjan för att undvika tillväxt av mikrobiella patogener.

Nyckelord: HACCP, livsmedelssäkerhet, biofloc, shrimp, landbaserad akvakultur

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Abbreviations

BFT	Biofloc Technology
CCP	Critical Control Point
CFU	Colony-Forming Unit
CL	Critical Limit
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSMS	Food Safety Management System
GAP	Good Aquaculture Practice
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
IDH	Dutch Sustainable Trade Initiative
LSP	Listed Specific Pathogen
NFA	Swedish National Food Agency
PCB	Polychlorinated biphenyls
PL	Postlarvae (shrimp growth phase)
PRP	Prerequisite Program
SLU	Swedish University of Agricultural Sciences, Sveriges Lantbruksuniversitet
SPF	Specific Pathogen Free
U.S.	The United States
WQM	Water Quality Management
WWF	World Wide Fund for Nature, Världsnaturfonden

1 Introduction

The global demand for aquaculture products is rapidly increasing. To supply the growing population, one can no longer rely on the seas and lakes alone to provide people with fish products. Aquaculture is predicted as the future for the fish industry and cultured shellfish in particular is predicted to take a most important part in feeding the world's population (Shumway & Rodrick, 2009). It is, however, desirable that contemporary and future production systems are developed towards environmental, social and economic sustainability (Simons, 2015).

Because of overexploitation, the supply from wild marine capture is no longer expected to increase. Thus, a big issue is how the aquaculture sector can meet the demand for supply when it is notorious for its negative environmental impact. Many aquaculture systems of today causes destruction of ecosystems, depletion of fresh water resources, salinity intrusion in ground water, mangrove removal, devastating land seizure for local communities and release of organic wastes and toxic effluents (e.g. overuse of antibiotics and pesticides), *inter alia* (Simons, 2015). Figure 1 presents FAO statistics on the globally increased cultured production of white-legged shrimp (*L. vannamei)*, the most common shrimp species today.

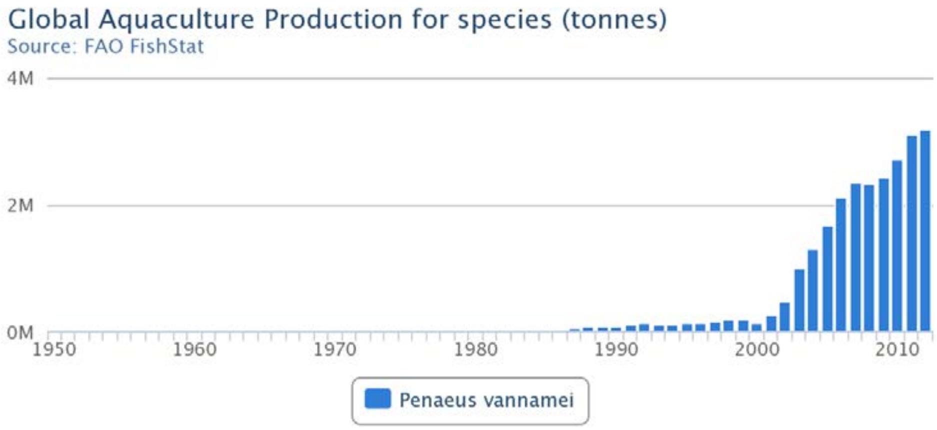

Figure 1. The global production of cultured *L. vannamei* increased rapidly from 194 000 tonnes in 1998 to 1 386 000 tonnes in 2004 and 2 297 000 tonnes in 2007 (Browdy *et al,* 2012). The main producer in 2004 was China with 700 000 tonnes. (Illustration: FAO, 2015).

To cope with the bad reputation of aquaculture, WWF and IDH in 2010 founded the now widely distributed Aquaculture Stewardship Council (ASC) standard aiming to manage the global standards for responsible and sustainable aquaculture (ASC, 2015). In Sweden, the national WWF organisation have given cultured shrimps “red light” in their guidance document for fish consumption, meaning that consumption is not recommended at all due to the negative environmental impact caused by tropical shrimp aquaculture (WWF, 2015). In year 2012, the non-profit organisation Naturskyddsföreningen launched a campaign called “Anti-Scampi” as an attempt to inform consumers about current circumstances regarding shrimp production (Naturskyddsföreningen, 2012). In 2015, the organisation claimed that even an ASC certification on tropical shrimp products is insufficient due to bad implementation (*ibid.*). However, the Swedish import of tropical shrimp has not been affected by the notorious reputation. Between 2006 and 2011, the import increased by almost 20 % (SCB, 2015).

The company VegaFish Bjuv AB has developed and initiated environmentally sustainable land-based processes for aquaculture production in Sweden by combining proprietary highly efficient production systems with methods where industrial waste heat is utilized to reach a tropical climate (VegaFish, 2015). As aquaculture is under increased scrutiny, VegaFish seeks to certify the production according to ISO 9000, 14000 and 22000. Because of the increased scrutiny and the need of having a holistic approach on the risk assessment and management, serious aquaculture producers of today must be proactive and take the time to identify all potential risks associated with their production. A globally established method to prevent food safety hazards within food production and processing is Hazard Analysis and Critical Control Points (HACCP), which is also a requirement in the ISO 22000 certification. The EU regulations of today states that every food business operator within food processing industries are required to develop a HACCP plan, a requirement which is expected to be extended to cover also primary productions.

1.1 Problem

Whether or not the EU commission incorporates a future expansion of the regulation on foodstuff hygiene (EC No 852/2004) for primary productions to establish HACCP plans, yet today it is encouraged to do so. Nevertheless, application of the HACCP principles is required to get an ISO 22000 certificate. The challenge in developing a HACCP plan for VegaFish’s shrimp production is that there are rather few or no data on similar production facilities to be inspired from, and even if there is, the HACCP principles is presumably not applied because it is not a requirement for primary productions. This settles the importance that VegaFish carefully investigates all potential food safety hazards in the HACCP plan.

1.2 Aim and delimitations

The aim of this study is to establish a HACCP plan for the land based production of white-legged shrimp (*L. vannamei*) to be distributed frozen and then fully cooked before consumption, operated by VegaFish AB. The HACCP plan covers both the research facility in Uppsala and the commercial facility in Bjuv. The aim of the HACCP system is to attain food safe for human consumption (food safety), why neither food quality nor shrimp safety (animal welfare) is included in this study.

In this report, the concepts of “shrimp”, “shrimp production” and “shrimp aquaculture” refers to shrimps grown in tropical habitats in the *Penaeidae* family, often referred to as “penaeid shrimps”

2 Literature review

Food safety and sustainability are in many ways related, where organisations at every step of the supply chain are working together on research and innovation to provide the population with safe foods. The fish farming industry faces an unprecedented demand for an increased production as well as requirements for more ecologically sound management. Following chapter presents what the literature can tell upon the topics of an innovative aquaculture production system aiming for sustainability, present food safety legislation and how they may meet to provide shrimps safe to consume.

2.1 Biofloc Technology

In the past years, interest in Biofloc Technology (BFT) has grown rapidly by being emerged as an “environmental friendly” system with a “green” approach, well suited for e.g. shrimp or tilapia production. The method constitutes a promising alternative to conventional aquaculture systems being both highly cost efficient and low in the use of natural resources (Emerenciano *et al.*, 2013). BFT is based on the concept of controlled cultivation of a microbial community within a culture media (water tank/pond). The microbial co-culture provides ecosystem services to the production unit by maintaining the water quality *in situ* through cycling of waste materials (e.g. nitrogen compounds) and by providing supplemental nutrition to the target crop (Browdy *et al.*, 2012).

BFT is a closed aquaculture system, meaning that the water exchange is minimised or close to zero, resulting in an optimised utilisation of both land and water resources and efficient prevention of nutrient leakage and environmental pollution where <1 m^3 water is required to produce 1 kg fish (*ibid.*). The recycling of water may also reduce the risks of pathogen introduction or escapement of foreign species (Emerenciano *et al.*, 2013).

In contrast to recirculating aquaculture systems where particulate matter is usually removed by filtration, the BFT concept encourages the microbial community to form particles within the culture media. The water tank is continuously mixed and aerated with oxygen, inducing bacteria, zooplankton, rest feed particles, organic polymers, dead cells and waste matter form macro-aggregates, i.e. *bioflocs*. These microbial flocs are irregular in shape and can grow to a size of 1000 µm, visible to the eye. The bioflocs are highly porous (up to more than 99% porosity), permeable to fluids and held together by physicochemical forces and polymer matrices composed of polysaccharides, proteins and humic complexes. The bioflocs constitute a nutrient-dense ecosystem in a nutrient-poor water environment, attractive to microorganisms which can graze from the inside or outside of the biofloc (De Schryver *et al.,* 2008; Browdy *et al.,* 2012).

Bioflocs constitute a nutrient dense biomass grown on animal excreta and nutrient waste material and can therefore serve as primary or supplemental feed to the target crop (e.g. shrimp), consumed actively or passively, available 24 hours a day. Furthermore, these macro-aggregates have been shown to contain essential amino acids and minerals, suggesting that these microbial communities can enhance the growth of the target crop and reduce feed costs (Emerenciano *et al.,* 2013). The ability to transform dissolved nitrogen into microbial proteins is further enhancing the advantages of the system. Formulated shrimp feeds contain high levels of protein, but a major part (up to 70-75%) of the nitrogen from the feed proteins are excreted to the water as uneaten feed or as metabolic waste products. Nitrifying bacteria within the bioflocs transform toxic ammonia which is potentially lethal even at low levels to most organisms to nontoxic nitrate compounds and high-quality proteins (Browdy *et al.,* 2012). A supplement of organic carbon (e.g. molasses) enhances the heterotrophic bacterial community and hence the water quality. A high C:N ratio stimulates algae and heterotrophic bacterial growth and thus the conversion of inorganic chemicals to new cell materials, proteins and cells, shown in figure 2 (Crab *et al.,* 2012).

The low water exchange in combination with the addition of simple sugars has raised doubts concerning potentially pathogenic bacteria getting a foot-hold in the BFT system. The number of studies on the topic is low, but there are suggestions that BFT can pose a new strategy for disease management (without usage of antibiotics) where the microbial community competes against microbiological pathogens for space, substrate and nutrients causing a limitation of their growth, also referred as a “probiotic effect” (*ibid.*).

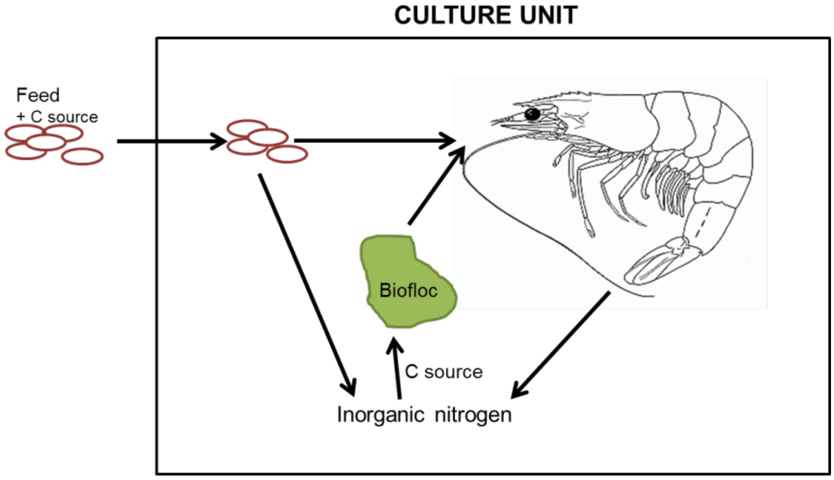

Figure 2. Schematic presentation of BFT, where bioflocs initiate their growth within the culture unit by using feed with a relatively low nitrogen content. The feed acts as a supplemental carbon source, primarily to the biofloc. Inorganic nitrogen waste and the added carbon source are consumed by the biofloc, producing microbial biomass (e.g. proteins) that can serve as alternative feed to the shrimps. Modified from Crab *et al.*, (2012). (Shrimp illustration: FAO, 2015).

2.2 Food safety control

The most basic expectation people have on food is usually that it is safe to consume. Food safety has been defined as the *"assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use"* (CAC, 2003). Food-borne illness and injury are at its best unpleasant - at worst, it can be fatal.

The global food market is complex, crossing national boundaries and cultures on a regular basis (Simons, 2015). Globally accepted standard systems and certifications can contribute to a food organization in its work to identify, control and prevent food safety hazards. Food safety management systems (FSMS) make up a reliable declaration of an organisation's ability to ensure safe foods towards customers and consumers. FSMS are in general applications of the laws and regulations regarding food and food safety (ISO, 2015).

2.2.1 ISO 22000

ISO 22000 is a series of standards that addresses food safety management, where ISO 22000:2005 consider overall guidelines towards food safety. To get an ISO 22000:2005 certificate, the food business operator is required to manufacture products fit for human consumption (Bergström & Hellqvist, 2006). The certification is structured in such a way that it is applicable for all actors in the food chain, from primary production to consumption, regardless of operating/business size

(ISO, 2015). This tailor-made approach minimises the risk of having "weak links" in the food chain, according to Færgemand (2008). The standard is built upon four mandatory key elements; (i) interactive communication, (ii) system management, (iii) prerequisite programs and (iv) the seven HACCP principles. Furthermore, the standard describes what the FSMS has to comprise but not *how* it should be implemented. It is up to each food business operator to figure out how to meet the requirements of the standard, based on individual qualifications. This "loosely held" framework makes it possible for all actors in the food chain to run the standard, an approach which supports the goal to ensure food safety from primary production to consumer (Bergström & Hellqvist, 2006). When ISO 22000 is operated through the whole food chain the traceability is substantially facilitated, and to recall to regulation (EC) No 852/2004 on the hygiene of foodstuffs, *"the traceability of food and food ingredients along the food chain is an essential element in ensuring food safety"*.

2.2.2 EU legislation on food safety

One of the fundamental objectives of the European food law is to maintain a high level of protection of human health (EC, 2002). Every food business operator within a member country of the European Union, all along the food chain has responsibility to produce and provide food fit for human consumption. One of the fundamental principles of the EU regulation (EC) No 178/2002 laying down the general principles and requirements of food law is that "*safe and wholesome food contributes significantly to the health and well-being of the citizens, and to their social and economic interest"* (General principle 1). Another principle is the aim of achieving free movement of food within the EU, which would never be reality if the food markets supplied hazardous foods. Food laws shall be based on preventive approaches through risk analysis in order to achieve a high level of protection of human health and life. The general implementation towards achieving food safety is based on the HACCP (Hazard Analysis and Critical Control Points) principles, which in its turn is based on the application of prerequisite programs (PRPs) for hygiene and sanitation standards (EC, 2004).

2.2.3 HACCP

The HACCP system is generally recognized as the major safety assurance system within a food production (Kanduri & Eckhardt, 2002). HACCP works systematically to identify and measure hazards aiming to ensure the safety of foods, based on credited science, once developed and provided by the Codex Alimentarius Commission (CAC/RCP 1-1969). More important than providing effective control mechanisms, the HACCP system is a risk management approach of safety control at all levels in a food operating business (Kanduri & Eckhardt, 2002, Garrett *et al.*, 2000). The HACCP system may therefore be regarded as an instrument or a tool to attain safe food products with high quality. Within the EU, all food processing operators (excluding primary productions) are required to develop, implement and maintain a HACCP system on a permanent basis (EC, 2004). The goal is to prevent potential hazards at the earliest stage possible in a food chain, through identification, prioritisation and minimisation of hazards.

The HACCP system is somewhat criticised for not being fully operable in primary productions because of the lack of effective ways to control identified CCPs such as cooking, drying or acidification *et cetera* make in processing operations (Sperber, 2005). According to Cerf *et al.* (2011) PRPs constitute the foundation for ensuring food safety through the supply chain, while the HACCP plan make out a complement in processing operations through the determination of CCPs. However, it has been suggested that the system is operable in shrimp aquaculture, including the production and handling of raw materials (Tookwinas & Keerativiriyaporn, 2004). As a matter of fact, a future extension of the EU regulation aims to include requirements also for primary productions to implement the HACCP principles. It is therefore encouraged that primary production operators apply these principles as far as possible already today (EC, 2004).

Establishment and verification of the HACCP system

Before developing a HACCP system, food business operators are encouraged to recognize some prerequisites for the hygiene and sanitation standard at the production plant. With sufficient PRPs in routine, the HACCP is likely to operate more efficiently by focusing on instant hazards associated with certain steps within a production (Kanduri & Eckhardt, 2002). The HACCP system is based on seven principles, shown in table 1. Procedure guidance on the establishment of a HACCP plan based on the seven principles is presented in Appendix 1.

Table 1. *The seven principles of HACCP, as described by Codex Alimentarius Food Hygiene Basic Texts (Bergström & Hellqvist, 2004).*

The seven principles of HACCP
1. Identify potential hazards and appropriate preventive measures (risk analysis)
2. Determine critical control points (CCPs)
3. Establish critical limits (CLs)
4. Establish monitoring procedures of the CCPs
5. Set corrective action to implement when a CCP is monitored
6. Establish a HACCP verification procedure
7. Establish procedures for record keeping and documentation

Official control, revision and approval of the HACCP plan and its verification are conducted by an appropriate competent authority (the Swedish National Food Agency or the local municipality). According to regulation (EC) No 852/2004, food business operators have by law responsibility to give the authorities sufficient information about their establishment, including production, processing and distribution (article 6, chapter II). For primary production operators, official control is conducted by the county administrative board (Länsstyrelsen).

2.2.4 Prerequisite programs (PRPs)

To get a HACCP plan to work efficiently, there is a need to clarify the relationship between good practice (aquacultural - GAP, manufacturing - GMP, hygienic - GHP) and HACCP (Jouve, 1998). The concepts of good practice are essential in a food control system providing basic environmental and operating conditions for food safety and quality. While the purposes of GAP and GMP primarily focus on quality aspects within a production, GHP solely focusses on the safety of products through hygiene and sanitation standards (Huss *et al.*, 2004). Thus, GHP are integrated in the basic requirements (the PRPs) and make the absolute foundation of food safety, see figure 3 (EU, 2005b). PRPs include infrastructural and equipment requirements, safe handling of raw materials and food, food waste handling, pest control procedures, sanitation procedures, water quality, maintenance of the cold chain and the personal hygiene, health and training, *inter alia* (EC, 2005b).

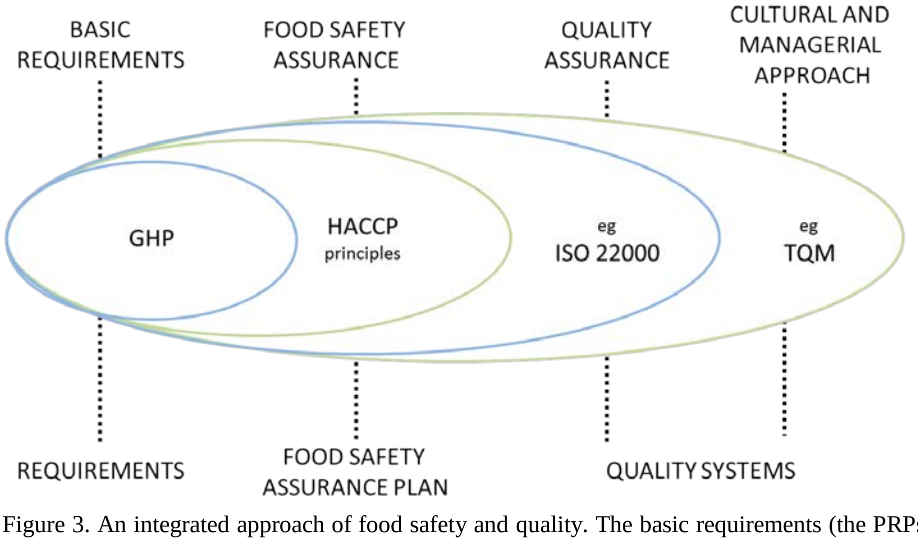

Figure 3. An integrated approach of food safety and quality. The basic requirements (the PRPs) makes the foundation for the food safety assurance (HACCP). Quality systems through international approved standards may then make an extended arm in the work for food safety. Inspired from Jouve (1998). *TQM = Total Quality Management*

2.3 Statistics on seafood-borne diseases

As for most food categories, the true incidence of diseases transmitted by seafood is unknown because of insufficient or non-existing reporting systems in both developing and developed countries (Huss *et al.,* 2004). The reliability of available statistical data is somewhat unclear due to a lack of differentiation between the wide range of production methods (e.g. aquaculture and wild-catch) and health care systems. However, statistics from FAO shows that the number of seafood-borne outbreaks from shellfish is much lower than for fish and molluscan shellfish. Oysters are well-known as high-risk food because of traditional reasons to eat them raw (Huss *et al.,* 2004). In table 2 showing statistical data from the U.S., shrimp is sorted under “other shellfish”. Reported cases of disease in the U.S. are usually caused by mishandling or cross contamination in retail service or at home (Otwell & Flick, 1995).

Table 2. *Food-borne disease outbreaks in the United States from 1990 to 1998 (Huss et al. 2004).*

Seafood group	**Outbreaks**	**Cases**
Fish	263	1661
Molluscan shellfish	66	3281
Other shellfish	**8**	**146**

2.4 Characterization of hazards in cultured crustaceans

In contrast to acquainted hazards associated with wild-caught shrimp products the intensive husbandry in aquaculture systems can pose new risks or hazards traditionally not associated with the sector. On a global scale shrimp represents one of the safest forms of muscle protein for consumption, still it is of great concern to regard the fact that all crustaceans have a tendency to spoil rapidly after harvest (Otwell & Flick, 1995).

The common case for some crustaceans, such as lobsters and crabs is to control their spoilage by keeping them alive until immediately before cooking. Shrimps, however, are killed soon after harvest or landing and the risk for contamination by spoilage microbes is by then increased. Shrimps have a neutral pH-value and high water activity which makes them highly perishable. The chemical composition with large amounts of free amino acids and high levels of muscle proteins make shrimps an excellent substrate to growing microbes (Brito *et al*., 2015). The endogenous microflora (that generally contains low levels of pathogenic microorganisms) and the external environment (i.e. mud, bird droppings and fish feed) or chemical run-off residues in outdoor production systems can be sources of contamination. Once the microbes find their way to the shrimps, they can grow very fast compared to any other type of food (Kanduri & Eckhardt, 2002). Some microbes found in shrimp may be pathogenic and pose a risk to public health (Brito *et al*., 2015). Sufficient processing such as freezing or cooking directly after harvest is therefore essential to ensure food safety (Adams & Moss, 2008).

In the development of a risk assessment potential food safety hazards will vary according to the system of culture (Reilly & Käferstein, 1997). The etiological agent of recorded seafood-borne diseases caused by shrimp and shrimp production indicates a slight variation depending on environmental conditions and geographic whereabouts. According to Tookwinas & Keerativiriyaporn (2004), the most common hazards in cultured shrimp in South East Asia include microbial pathogens, veterinary drugs and aquaculture chemicals or other environmental contaminants. In the US, a majority of the seafood-borne diseases has been traced to microbiological contaminants caused by e.g. temperature abuse during processing (Huss et al., 2004; Otwell & Flick, 1995). According to Jones (2009) enteric viruses, pathogenic *Vibrio* spp. and to lesser extent fecal-borne bacterial pathogens constitute the main causes of shellfish-borne diseases.

2.4.1 Biological hazards

Despite the scarcity on statistical data of shellfish-borne diseases there are indications that bacteria and viruses are the main biological disease causing agents, probably because shrimp pose a nutritious substrate to these organisms (Huss *et al.,* 2000). A convenient way to organise seafood-borne pathogenic bacteria is to sort them into groups according to their ecology and origin (listed in table 3). Huss *et al.,* (2004) suggests following microbial classification:

- The aquatic environment
- The general environment
- The human/animal reservoir

The level of pathogenic bacteria from the aquatic environment is generally low in or on shellfish, but may constitute a natural part of the micro flora. Former studies have recorded only low or very low concentrations of *i.e.* pathogenic strains of *Vibrio* spp. from the aquatic environment in cultured shrimp (Brito *et al.,* 2015; Daalsgard *et al.,* 1995; Daalsgard *et al.,* 1996) Pathogens from the general environment present on shellfish may be a result of unavoidable contamination, but are rare on or in shrimps. In contrast to pathogens originating from the aquatic and the general environment, where the mere presence is of no safety concern, only a minor presence of pathogens from the human/animal reservoir can cause serious safety issues, especially for products eaten raw (Huss *et al.,* 2004). For pathogenic *E. coli* O157 the minimum infective dose can be as low as 10 cells (FDA, 2011). The main preventive measure to avoid contamination of pathogens from the human/animal reservoir, e.g. pathogens normally not present in shrimps, is the application of GHP. However, some of these bacteria are toxin producing pathogens (i.e. *Staphylococcus aureus*), thus growth in the product is required to cause risk of disease (Huss *et al.*, 2004).

Table 3. *Seafood-borne pathogenic bacteria in selection and their mode of action of disease. It must be emphasized that all genera of pathogenic bacteria may contain non-pathogenic strains (modified from Huss* et al.*, 2004).*

Origin of pathogen	**Organism**	**Mode of action of disease**
Aquatic environment	*Vibrio parahaemolyticus, V. cholerae*	Infection
General environment	*Clostridium botulinum* Type A, B	Intoxication
	Listeria monocytogenes	Infection
Human/animal	Salmonella	Infection
reservoir	*E. coli*	Infection
	Staphylococcus aureus	Intoxication

In the risk management of controlling viral pathogens a setup of Listed Specific Pathogens (LSP) is a convenient tool, mainly used for viral shrimp pathogens (Schwarz, 2007). As for bacterial pathogens, the statistical data on what specific types of human viruses that have been associated with shellfish vary. The European Commission and Codex Alimentarius mention Norwalk-like viruses in their regulations and guidance documents (EU, 2005a; CAC, 2003), whereas Jones (2009) also takes i.e. Rotaviruses, Enteroviruses, Caliciviruses and Hepatitis A into account.

Regulated microbiological criteria for fishery products in the EU

Since food-borne diseases in humans are most commonly caused by microbiological hazards, the European Commission have laid down Regulation (EC) No 2073/2005 *on microbial criteria on foodstuffs*, aiming to establish a harmonised safety approach on the presence and acceptability of certain pathogenic microorganisms in food (EC, 2005a). It is appropriate to define the microbiological acceptability of a food production based on the criteria of the regulation, and they can later on be used for validation and verification of the HACCP procedures.

For shellfish the regulated criteria are delimited to shelled and shucked products of cooked crustaceans, where *E.coli* (no more than 10 cfu/g) and coagulase-positive staphylococci (no more than 1000 cfu/g) are the pathogens of concern and unsatisfactory test results requires improvements in production hygiene. For cooked crustaceans and molluscan shellfish, Salmonella is stated as a pathogen of concern if found in products placed on the market during their shelf-life (*ibid.*). For frozen fish products to be cooked before consumption there are no microbiological criteria, neither any labelling requirements regarding information to the consumer of the need for thorough cooking prior to consumption, as there is for certain meat products. However, in Swedish there is a special control program based on regulation (EC) No 2160/2003 *on the control of Salmonella and other food-borne zoonotic agents* aiming to eliminate the risk for contamination by Salmonella that every food business operator has to comply with (SJV, 2015). Moreover, it is stated that there are currently no available scientific data to support specific criteria for pathogenic *Vibrio parahaemolyticus* and *vulnificus*, although they are common in seawater and sometimes also in seafood. It is, however, likely that a code of practice for *Vibrio* criteria will be defined in future establishments of the regulation (EC, 2005a).

2.4.2 Chemical hazards

A chemical hazard is a substance found in the product which is poisonous or allergenic at a certain concentration. The risk of acute chemical intoxication is in general rare, but long-term, low-level exposure to chemical contaminants may be associated with serious diseases which also can be hard to trace. Chemical contaminants with a potential toxic effect in shrimp production can be inorganic chemicals (e.g. sulfites used to prevent polyphenoloxidase activity, or heavy metals), organic compounds (e.g. PCBs, pesticides) or processing related chemical compounds such as antibiotics or hormones (Huss *et al.*, 2004; Kanduri & Eckhardt, 2002). Chemical contamination of shellfish is not a significant hazard except for those circumstances when the polluting agents reach levels high enough to pose a substantial risk in aquaculture (Huss *et al.*, 2004). Monitoring and detection of chemical contaminants can be challenging because of the need for expensive specialized laboratory analyses. Another challenge is the lack of preventive measures for unexpected and novel contaminants in aquaculture (Egiraun *et al.*, 2015).

Regulated sulphite criteria for fishery products in the EU

The chemical agent sodium metabisulphite (E 223) is commonly used in shrimp processing as a preservative to inhibit microbiological growth and prevent the browning quality defect called blackspots. However, sulphites are allergens, posing a particular risk for asthmatics (SLV, 2015a). According to Regulation (EC) No 1169/2011 on provision of food information to consumers, *sulphites at concentrations of more than 10 mg/kg or 10 mg/litre in terms of the total* SO_2 in the final product must be declared (EC, 2011).

2.4.3 Physical hazards

A physical hazard is defined as any foreign material in seafood products which is not normally associated with it and that is potentially harmful (Kanduri & Eckhardt, 2002). A foreign material in seafood products can be classified as non-food safety hazards (e.g. filth) and food safety hazards (e.g. glass, metal or hard plastic). The harmful effect of physical hazards can be injury, choking or broken teeth. The source of such a hazard is usually easy to trace and identify (Huss *et al.*, 2004).

2.5 Risk assessment in Biofloc shrimp production

The following example aims to explain and briefly evaluate the complex elements which needs consideration in order to attain food safety within a closed land-based BFT production of white-legged shrimp (*Litapenaeus vannamei*), to be distributed and stored frozen and fully cooked before consumption.

2.5.1 Hazards of concern

The Swedish National Food Agency (NFA) has established a list of microbiological and chemical hazards (table 4) associated with certain foods. All listed hazards are not always significant, but the NFA recommends that they are taken into account in the risk assessment of any product within the food category.

Table 4. *Hazards associated with shellfish products (SLV, 2015b).*

Type of food	Microbiological hazards	Chemical hazards
Shellfish, including	Salmonella	Allergens
bivalve molluscan	*Shigella*	Algae toxins
shellfish,	*Staphylococcus aureus*	Chemical residues
shellfish products	*Listeria monocytogenes*	
	Vibrio spp.	
	EHEC/VTEC	
	Norovirus	
	Hepatit A virus	

Despite the fact that shrimps are prone to spoilage, the hazard analysis of a shrimp production can be reasonably uncomplicated (Huss et al., 2004). In a recent study conducted in Brazil by Brito et al., (2015), the microbiological quality in *L. vannamei* BFT cultured shrimp was examined. The study covered the presence of Salmonella spp. and coagulase-positive Staphylococci for which legal limits are established, and *Vibrio* spp. and coliforms because they represent a potential public health risk in Brazil. The study aimed to examine whether the low water exchange in BFT system may stimulate accumulation of pathogenic organisms. The analysis results detected neither coagulase-positive Staphylococci nor Salmonella, and only low concentrations of *Vibrio* and coliforms, indicating that shrimps produced in the BFT system were acceptable for human consumption. When Daalsgard et al., (1995) analysed 158 samples from a conventional outdoor production in Thailand, including samples from water, sediment, shrimp and formulated feed, no Salmonella was found, indicating that these bacteria might not be that common in shrimps.

2.5.2 Pre-harvest

In a generalised flow diagram model, Reilly & Käferstein (1997) identifies production site selection, water supply, production method and feed input as four typical pre-harvest CCPs in an aquaculture production, shown in figure 4. A major driving force should thereby be prevention of chemical and microbial contamination and to control the pathogen growth (Kanduri & Eckhardt, 2002). The main pathways for pathogen introduction indicate to be infected postlarvae and/or incoming water (Brito *et al.*, 2015; Emerenciano *et al.*, 2013). The risk of water contamination by chemical residues such as antibiotics, pesticides or herbicides is minimised by using water provided by the municipality (Vattenförvaltningen, 2009), since also drinking-water distributors are obligated to implement permanent procedures based on the HACCP principles according to regulation (EC) No 852/2004 (SLVFS 2001:30). Furthermore, changes in water quality (e.g. dissolved oxygen, pH and salinity) within the production site can lead to stressful conditions and growth reduction. If the stress applied during the growout period is minimised or eliminated the shrimp's susceptibility to disease can be reduced. A good water management protocol can keep the biofloc at good health and thereby contribute to the safety of the final products (D'abramo *et al.*, 2009). Brito *et al.*, (2015) showed that the concentrations of coliforms from contaminated postlarvae decreased during the growout.

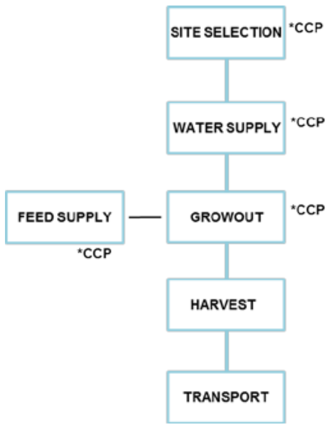

Figure 4. Model flow diagram for aquaculture production, with typical CCPs, modified from Reilly & Käferstein (1997).

Feed input

Additional feed should be distributed by reliable sources to prevent contamination by pathogens or unwanted chemical in the feedstuff (D'abramo *et al.*, 2009). The improvement of biosecurity in BFT by *in situ* recycling of waste matter and reduced use of additional feed can decrease the risk of pathogen introduction, thereby enhancing the food safety (Emerenciano *et al.*, 2013).

Harvest

During harvest the number of personnel and their activity is likely to increase, thereby requiring sufficient PRPs to prevent contamination of shrimps. Personnel should have adequate training in basic hygiene, and all tanks and equipment used during harvest must be clean and sanitised according to the PRP. Ice and/or water used for chill killing should be properly handled to prevent contamination. The temperature of the water should be monitored to confirm adequate cooling (D'abramo *et al.*, 2009). The chill killing procedure should be kept long enough to decrease the core temperature of all shrimps to 1-2 °C, but no more than 20 minutes because of the risk of degrading the texture of the tail. Salt added to the water can speed up the process. To confirm adequate cooling, temperatures of shrimp tail core should be measured (Tidwell & Coyle, 2011). Addition of sulphite agents must be carefully monitored to prevent undesirable concentrations in the final product, unless the allergen is included in the product declaration (Kanduri & Eckhardt, 2002). According to Tidwell & Coyle (2011), the shrimps can then be held on drained ice two days before the quality and safety is decreased.

2.5.3 Post-harvest

Pathogenic microorganisms may be present on raw shrimps from the natural flora and/or as a result of cross contamination from mishandling. If the temperature are kept low it is unlikely that the pathogens will grow nor cause any food-borne disease, especially since the shrimps is to be cooked before consumption. Also microbiological parasites, if present, will be killed by household cooking. It is likely that pathogens in the final product are derived from the culture media or from human mishandling during harvest (i.e. bad implementation of GHP) (Rosengren, 2015). This indication is somewhat verified by Amagliani et al. (2012); Huss *et al.* (2004) and Hatha *et al.* (1997), suggesting that potential pathogens of concern in shrimps are mainly Salmonella, *E.coli*, *Vibrio* or Staphylococci. Despite that these bacteria are potential hazards and may be subject for regulatory sampling, their mere will most likely not be CCPs since the products are not to be eaten raw (Otwell & Flick, 1995). Most hazards related to consumption of frozen shrimp to be cooked before consumption can therefore be controlled by the application of PRP and HACCP. The freezing conditions during distribution and storage and the terminal heat application puts shrimp in a low risk category of food. Nevertheless, the importance of consumer information about the risks of eating raw shrimp should be carefully addressed when marketing the product; otherwise the absence of CCPs for identified hazards may pose a risk of causing food-borne disease (Huss *et al.*, 2000).

3 Materials and methods

This chapter describes the approach of the master thesis project's research design. The present qualitative study was conducted at VegaFish AB shrimp production facilities in Uppsala and Bjuv.

3.1 Research approach

The purpose of the study was to establish a HACCP plan for the shrimp production in order to meet the regulations of the ISO 22000 certification. According to current versions of the EU regulations a primary production is not required to adopt a HACCP plan, yet it is necessary in the implementation of the ISO 22000 standard.

The study used is a descriptive qualitative approach, a combination of data collection, interviews, analysis and discussion (Sandelowski, 2000). The results have been interpreted and analysed with regards to the findings in the literature review.

The main stages of a qualitative study are presented in table 5, based on Bryman & Bell (2011). With respect to the importance of attaining a trusted level of food safety, the conclusions regarding potential food hazards within the production has been carefully discussed with VegaFish AB.

Table 5. *Overview of the main steps in a qualitative research study.*

Stages in a qualitative study
1. General issues
2. Selection of relevant sites and survey people
3. Collection of data
4. Interpretation of data
5. Conceptual and theoretical work
6. Report on the results and conclusion

3.2 Literature review

A literature review collects and reports what has already been published on a particular topic by researchers, as an introduction to a research report (Taylor, 2015). The literature review is normal procedure and should be the starting point in any research project to avoid reproducing/overproducing studies with similar results, or just to avoid making previous mistakes.

The number of scientific articles on food safety and/or HACCP in closed BFT systems such as the one approached in this study is rather limited. Therefore, relevant scientific literature on the different topics (mainly development of HACCP plans, food hazards in shrimp production and the concept of biofloc technology systems) has been combined with the empirical data. This master thesis may be an input to the literature collection on how to attain food safety within this certain kind of production.

3.3 Search strategy

The PubMed, Web of Science, ProQuest and Google Scholar databases was searched for English language articles, using the search terms *biofloc*, *HACCP*, *food safety*, *control* and *aquaculture* in many different combination, where relevant articles and reviews were selected. Textbooks were obtained by the SLU Library and books.google.com. Legislation, regulations and guidance documents were primarily obtained through institutional websites of EU, FAO and FDA.

3.4 Empirical approach and collection of data

Where the literature review collects knowledge of what is known on the topic of today's date, the empirical research gains new knowledge by observations and experiences. The empirical approach can be used to answer specific research questions. Furthermore, the empirical approach normally has one main theory which the researcher tries to explain with help of the observations or experiences (Moody, 2002). The research question within this study was formulated as: *"What are the critical steps in the shrimp production to attain food safety and how can the HACCP be planned to prevent presence of potential hazards?"*

Since this was a qualitative study, the data collected is in form of text and figures, written and drawn from observations and documentary evidence. The empirical data was collected during meetings with VegaFish AB.

3.5 Case study

A case study is an in depth study for understanding the complexity and the specific nature a certain case may show (Bryman & Bell, 2011). Theories developed by the case study method are likely to contribute to important, novel conclusions, if carried out in a proper manner (Eisenhardt, 1989). The research method is commonly used in social sciences when the main research question is "how" or "why", which is the case in this project where the researcher seeks answer to the question how to attain food safety within the shrimp production. According to Yin (2014), *"a case study investigates a contemporary phenomenon (the "case") in its real-world context"*. This may be of advantage when the relations between the case and the context are not clearly evident. The case study has been carried out with the main steps of qualitative study from table 5 as inspiration.

3.6 Ethical aspects on food safety

As long as people will depend on global or local food markets for their satiety and safety, food and ethics will have a close connection. When it comes to the development of a HACCP plan, the main assurance of food safety, it is of great importance to ensure that all potential hazards are considered. Food ethics should always include production of safe high value foods, professional standards, continuous development, sustainability and honesty (Sikora *et al.,* 2013). The ethics of providing safe foods can furthermore be regarded as a risk minimisation approach to a company.

4 The empirical study

In this chapter observations from the study are given. After a brief presentation of the VegaFish company, the flowchart of the shrimp production is presented step by step in figure 5. The observations and conclusions drawn from the flowchart then form the basis for the risk assessment, hazard analysis and established HACCP plan presented in next chapter.

4.1 Presentation of VegaFish

The Swedish company VegaFish AB in cooperation with its strategic partner SLU and Findus Sverige AB has initiated an innovative project aiming to develop proprietary, highly cost efficient, organic, environmentally sustainable systems for shellfish and fish farming. The culture method is based on the scientifically proven system of Biofloc Technology (VegaFish, 2015).

The production system is built upon the concept of utilizing industrial waste energy that is converted to heat, creating a tropical acclimatisation necessary for the shrimp production. The system is classified as intensive, where the harvest may yield up to four times more per unit area compared to conventional systems. The production is held within closed land-based tanks with a water exchange close to zero (*ibid.*). Effluent water that is not set back into the system is discharged by a special municipal water treatment system according to current environmental legislation (SFS 1998:899). The company aims to get certificates of the ISO 9000, ISO 14000, ISO 22000, ASC and KRAV standards for their production. The frozen, unpeeled shrimps will be marketed at the general food retailer market.

4.1.1 The VegaFish food safety management

VegaFish has an ongoing work with the ISO standards and ASC certificate, which in many ways touches upon the topic of food safety. The PRPs are developed in parallel with this master's thesis project, where the efficiency of the presented

HACCP plan relies on the sufficiency of the self-monitoring processes (GHP/GMP) included in the PRPs.

4.2 Biofloc preparation

Municipal drinking-water is converted to an “artificial” sea water environment, by addition of specific biofloc components (chosen to resemble the natural flora of sea-water). The living components of the biofloc, i.e. bacteria and alga, are purchased from reliable sources. The probability that human pathogens would be present in is fairly low since the biofloc components likely competes against pathogens and therefore may have a negative impact on their vitality and survival. The inorganic components, e.g. salt and water quality chemicals are also purchased from reliable sources.

4.3 Operational flow chart

All steps involved in the production, from reception of shrimp postlarvae to cold storage after harvest through quarantine and growout is studied in sequence in the hazards analysis (chapter 5) and presented in a short flow chart in figure 5. A list of specific human pathogens (LSP) concerning the VegaFish production is presented in Appendix 2.

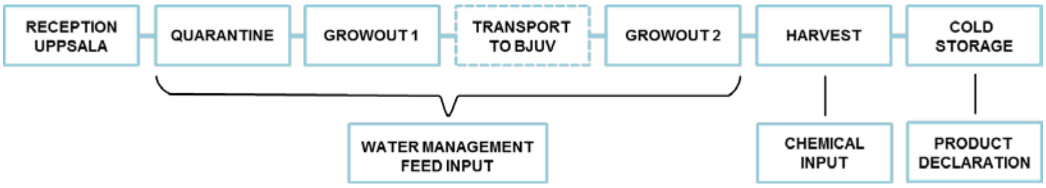

Figure 5. Schematic presentation of the production flowchart. A detailed flow chart is presented in Appendix 3.

4.3.1 Water quality management

The water quality management (WQM) procedures make an important part of the production line, aiming not only to maintain a stable culture in the biofloc but also to avoid introduction of human pathogens into the system. Tank water is monitored once every week for a range of specific pathogens during quarantine and growout phase, including human pathogens such as *Vibrio*, *Pseudomonas* and bacteria of the *Enterobacteriaceae* family (i.e. Salmonella and *E.coli*). During the growout phase also shrimp samples are taken. When test results indicate presence of unwanted microorganisms an alerting system is triggered for the specific contaminated tank, causing the personnel to take immediate action. The less stress applied to the shrimps, the less susceptibility to disease or other factors that can adversely affect the quality of the product. If depletion in shrimp growth health occurs due to pathogen introduction, the treatment includes addition of competitive "probiotic" microorganisms. No antibiotics are used since it will cause a biofloc collapse. The WQM is furthermore a substantial part of the PRP, where facility, tank and equipment hygiene is fundamental to avoid contamination.

4.3.2 Reception

Postlarvae shrimps of *Litapenaeus vannamei* (white-legged shrimp), are purchased from a brood stock in Florida, U.S. Imported shrimps are delivered with a certificate of origin and health, verifying that all of the brood stock originates from a Specific Pathogen Free (SPF) breeding center and that the shrimps are tested for important shrimp viruses, including i.e. Yellow Head Virus and White Spot Syndrome. The brood stock is reared in a closed BFT system. During the journey to its final destination in Sweden, the consignment of shrimps is veterinary examined for detection of listed specific pathogens (LSP) in terms of viruses pathogenic to shrimps, at least once at every fare-stage. On the arrival to the facility in Uppsala, the facility manager receives and signs the certificates of origin and health.

4.3.3 Quarantine

After the reception the shrimps are transferred to quarantine basins which also work as their nursery. The quarantine phase lasts one week and is labor intensive, requiring veterinary examinations and almost 24 hours a day supervision to make sure that the shrimps acclimatise to their new environment. The water salinity is reduced from 3,5 % to 1,5 % upon the arrival at a gradual conversion. In case the monitoring alert system indicates presence of LSP during quarantine in a certain quarantine basin, VegaFish does an individual evaluation of the contaminated basin and decides whether or not to terminate the quarantine procedure and discard the larva or treat it with probiotics, a preventive measure to avoid introduction of pathogens to the biofloc. A likely source of contamination could be the water the larva is held in during transport.

Feed input

During the quarantine and growout phases the shrimps are fed with fish feed and the biofloc with carbon-rich molasses, provided by reliable sources from feed manufacturers, also complying under the European food safety law. The feeds are stored cool and dry, isolated from the tropical temperatures in the tank areas.

4.3.4 Growout 1, transport and growout 2

After the quarantine phase the shrimps are transferred to growout tanks, where they are kept for approximately five weeks. Temporarily they are fed with shrimp feed, but the primary source of nutrition will during the growout eventually change from formulated feed to biofloc. Hence the monitoring of the biofloc health will be of great importance. After five weeks of growth the shrimps are transported to the facility located in Bjuv, where they continue to grow for approximately six weeks, until they reach their adult stage. The WQM routines proceed in Bjuv. During the transport, the stress applied to the shrimps is minimised due to a slight decrease in water temperature, which will lower the metabolic activity. GHP routines prevent the risk of contamination by pathogens. Feed input and water quality management is the same during the growout phases as during the quarantine phase.

4.3.5 Harvest

During harvest, the tank is drained and the shrimps are transported to a clean harvest tank. The shrimps are then separated from the biofloc and transported on a conveyor belt to a mobile basin with salted 0 °C clean water and sodium metabisulphite. The water is kept flowing to rinse away remaining biofloc from gills and legs, and the cold temperature kills the shrimps within 2 minutes. The chill killing procedure is kept for a maximum of 20 minutes to ensure that core temperature of all shrimps is 1-2 °C. At this time, the shrimps are manually tucked from the mobile tank to clean storage boxes. The boxes have to be transported to a cold storage within 10 minutes, or else spoilage bacteria may start to grow. The harvest is labor intensive and requires that the personnel are well trained in GHP to avoid contamination of pathogens from the human/animal reservoir. The separated biofloc and its culture media are monitored and stabilised until next batch of shrimp larvae is ready to grow in it.

Chemical input

To prevent black spots caused by enzymatic activity on the final product, sodium metabisulphite (E223) is added to the chill killing water tank.

4.3.6 Cold storage

The storage boxes in which the shrimps are kept in until next food business operator, Findus Sverige AB receives them are stored in cooltainers with a temperature of 2 °C, for no more than 24 hours.

Product declaration

Product information declaring the ingredients (shrimps and sulphite) and that VegaFish comply with current legislation is included in the hand over stage, according to the PRPs. It is recommended that cooking instructions are included in the final product declaration.

5 Results: the HACCP plan

Except from the author of this document, the HACCP team included the VegaFish CEO and the two facility managers, whom all together cover the whole range of specific knowledge needed to develop the HACCP plan. The description of the product and instructions for intended use are presented in table 6. The detailed production flow chart presented in Appendix 3 was confirmed by the multidisciplinary HACCP team, resulting in the hazard analysis in table 7. For the identified CCP control measures, critical limits, monitoring procedures, corrective actions, record keeping and verification procedures are presented in the HACCP plan in table 8. VegaFish is responsible that all personnel are aware of the identified hazards and the CCPs, and to delegate the monitoring and preventive measure procedures if needed. Training of the PRP procedures is essential for the food safety.

Table 6. *Full description of the product*

Product description	
Composition	Shrimp (*L. vannamei*), Sodium metabisulphite
Processing	Treated with metabisulphite in water for a maximum of 20 minutes, in 0 °C salted water during harvest.
Packaging	Packed in storage boxes
Storage and distribution conditions	Product to be stored in 2 °C cold storage
Required shelf-life	24 hours in 2 °C cold storage
Instructions for use	
a. By a further processor or retailer	a. Keep cold-chain
b. By the consumer	b. Product to be cooked before consumption
Any microbial and chemical criteria applicable	Product not sterile

Firm name:	VegaFish Bjuv AB	Product description:	White-legged shrimp *L. Vannamei*
Firm address:	Marsta Rosenlund 125, 755 93 Uppsala	**Storage and distribution:**	Frozen
Date:		**Intended use:**	Product must be fully cooked before consumption
Signature:			

Table 7. *Hazard analysis.* SPF – Specific Pathogen Free, LSP – Listed Specific Pathogen

HAZARD ANALYSIS					
(1) Processing step	**(2) Potential hazard introduced or controlled**	**(3) Is the potential hazard significant? (Reasonably likely to occur Yes/No)**	**(4) Justification for inclusion or exclusion as a significant hazard**	**(5) Preventive measures of the significant hazard**	**(6) Critical Control Point (Yes/No)**
Biofloc input	Biological - Presence of LSP	Yes	Biofloc components may contain detectable LSP	Controlled by PRPs, in which biofloc components are purchased from documented reliable sources.	No
	Chemical – *None identified*				
	Physical - *None identified*				
Water quality management, WQM *(Quarantine + Growout)*	Biological – Water or waterborne particles may contain detectable LSP	No	Water controlled by the municipality WQM chemicals may contain detectable LSP	Controlled by PRPs, in which WQM chemicals are purchased from documented reliable sources.	No
	Chemical – *None identified*				
	Physical - *None identified*				
Pre-harvest					
Reception - Outsourced shrimp postlarvae	Biological – Presence of LSP	Yes	Shrimp PL may contain detectable LSP	Controlled by microbial quality monitoring (PRPs)	No
	Chemical – *None identified*				
	Physical - *None identified*				
Feed input *(Quarantine + Growout)*	Biological – Presence of LSP from - Formulated biofloc feed (simple sugars) - Live shrimp feed (biofloc) - Formulated shrimp feed	Yes	Live shrimp feed may contain detectable LSP Formulated feed may contain detectable LSP	Controlled by WQM Controlled by PRPs, in which feeds are purchased from documented reliable sources.	No
	Chemical – *None identified*				
	Physical - *None identified*				

Quarantine	Biological - Presence of LSP	Yes	Shrimp PL may contain detectable LSP	Controlled by WQM and quarantine procedures	No
	Chemical – *None identified*				
	Physical - *None identified*				
Growout 1 & 2	Biological - Presence of LSP	Yes	Shrimp or water may contain LSP	Controlled by WQM procedures	No
	Chemical - *None identified*				
	Physical - *None identified*				
Harvest					
Transport to harvest tank	Biological – Presence of LSP	Yes	Potential pathogenic bacteria can be associated with insufficient GHP	Controlled by PRPs	No
	Chemical – *None identified*				
	Physical - Foreign matter	No			
Transfer to chill killing basin	Biological – Presence of LSP	Yes	Potential pathogenic bacteria can be associated with insufficient GHP	Controlled by PRPs	No
	Chemical - *None identified*				
	Physical - *None identified*				
Chill killing In salted 0 °C water, with sodium metabisulphite,	Biological – Presence of LSP	No	Low temperatures in tank		
	Chemical - Sulphiting agents	Yes	Sulphiting agents are known to cause allergic type reaction	Controlled by product declaration	No
	Physical - *None identified*				
Post-harvest					
Packing in storage boxes	Biological – Presence of LSP	Yes	Time/temperature abuse during the packaging may result in growth of potential pathogens	Storage boxes must be transferred to cold storage within 10 from the moment the first shrimp is added, to avoid pathogen growth.	Yes
	Chemical - *None identified*				
	Physical - *None identified*				
Cold storage Storage boxes in cooltainer at 2 °C, maximum 24 h	Biological – Presence of LSP	Yes	Thermal abuse can result in pathogen growth.	Controlled by PRPs Product to be cooked before consumption	No
	Chemical - *None identified*				
	Physical - *None identified*				
Product declaration	Biological – *None identified*				
	Chemical – Sulphiting agents	Yes	Sulphiting agents are known to cause allergic type reaction	Proper label declaration on treated product	No
	Physical - *None identified*				

Table 8. *HACCP plan*

HACCP PLAN									
(1) Critical Control Point (CCP)	**(2) Significant hazard**	**(3) Critical Limits of the preventive measure**	**Monitoring**				**(8) Corrective Action**	**(9) Records**	**(10) Verification**
			(4) What	**(5) How**	**(6) Frequency**	**(7) Who**			
Packing in storage boxes after chill-killing	LSP presence/growth due to time/temp abuse	Shrimp storage box must be kept in cooltainer within 10 minutes from that the first shrimp is transferred from chill-killing basin to box	Time	Set time for 10 minutes (alarm clock)	Every new storage box	Harvest personnel	Discard shrimps which are kept in room temp for more than 10 minutes	Harvest time logs	Record review for every total batch

6 Discussion

Before any conclusions concerning the food safety of the VegaFish shrimps is drawn, it would be necessary to remind the reader about some starting-points regarding shrimp culturing. Frozen shrimps to be cooked before consumption are, independent on country of origin, production method or its tendency to spoil rapidly after harvest, classified as low risk food. These circumstances might implicate that food safety is fairly uncomplicated to attain. Furthermore, the biofloc system with its low water exchange, limited use of chemical components and naturally "probiotic" effect might appear to be safer from a food safety point of view than many other aquaculture methods. Nevertheless, a range of potential hazards are emphasized in the literature, including chemical compounds and microorganisms, primary from the human/animal reservoir.

There are primarily four inputs to the production; the postlarvae shrimp and accompanying water, the biofloc and WQM composites, the feeds and the product quality enhancing sodium metabisulphite. Neither of the inputs is identified as CCPs. There are a number of potential significant hazards in the hazard analysis in table 8 that may be regarded as Control Points. However, for a potential significant hazard to become a *Critical* Control Point, the preventive measure must be instantaneous to prevent the hazard. Chemicals, feeds and biofloc composite inputs and their hygiene standards are therefore covered by the PRPs. If they would have been identified as CCPs, monitoring of the microbiological quality would be needed for every new batch/delivery. This is labour-intensive and not really feasible in a primary production. Also, the food law is designed in such a way that feed, chemical and biofloc producers should supply products that comply with the food safety regulations – that is that they do not contain hazardous compounds. According to the PRPs framework, VegaFish needs to make sure that their inputs are provided from reliable sources which are complying with current food safety regulations, i.e. by asking producers/distributors for any documentation or certificates regarding the food safety of the company or their products. This documentation of the safety compliance should also include some sort of long-term guarantee.

6.1 HACCP - not a legal requirement, yet necessary

While the HACCP system is a tool to prevent hazards and establish control systems rather than final-product testing, the PRPs are designed to facilitate the successful application and implementation of HACCP. The PRPs should be well established in the daily operation and verified prior to application of HACCP. Therefore, the PRPs make the foundation to HACCP by preventing hazards within the production. In VegaFish's case as a primary production, there are to this day no legal requirements on the application and implementation of HACCP, yet the goal to attain an ISO 22000 certificate makes it necessary. The certificate does also show that VegaFish has good intentions regarding their production towards the consumers. The aquaculture sector has a notorious reputation for being environmentally unsustainable. VegaFish will with the ISO and ASC certificates in their hand prove that their production has joined the run for sustainable food production.

The production is in many ways innovative, being i.e. closed, land-based and recirculating. The microflora of the aquatic and general environment in Sweden may differ from other geographical sites where shrimps are commonly cultured. The use of chemicals is low and no antibiotic or growth enhancing compounds are used within the production. Therefore the geographical site can pose new potential hazards not traditionally associated with similar aquaculture production systems. This makes the application of HACCP not only a tool to attain food safety but also a securing measure to examine the characteristics of this particular production. It has been a challenge to pinpoint the particular hazards because of the scarcity on previous research on the topic and information from the authorities (i.e. the lack of EU-regulated microbiological criteria for frozen shrimps to be cooked before consumption). The HACCP plan presented in this document is therefore created to the best of ability, based on observations and the literature review. Most important of all, the HACCP plan is a living document which is supposed to be reviewed continuously.

6.2 Food safety of the shrimp production

The risk for contamination by hazardous compounds is reduced by using municipal water, which is probably the safest source of water available. If the municipal water would be contaminated it would be out of VegaFish's control and special actions would therefore be needed. A risk minimising factor in this production is that the system is closed which is a preventive measure against external potentially ecosystem disturbing contaminants (e.g. from bird droppings and rain). By keeping the tanks closed, monitoring of the biofloc composition is facilitated. Since the "sea-water" in the tanks is made artificially, the probability of aquatic environment pathogens is reduced. However, some *Vibrio* spp. are always associated with tropical shrimp and their detection is expected. The only likely source of contamination by *Vibrio* is thereby the water the shrimp postlarvae are transported with from the distributor to Uppsala.

The BFT system is designed to mimic the nature in a controlled way and the biofloc composition is designed in such a way that it should be able to compete with pathogenic bacteria, toxin producing alga and parasites. The high C:N ratio favours heterotrophs and decreases the growth of autotrophic microorganisms such as toxin-producing green alga. Nevertheless, prevention of pre-harvest *contamination* by naturally occurring pathogenic bacteria may be difficult because there is always a risk that they are present in the aquatic or general environment. If, however, a pathogen would gain a foothold in the biofloc, the weekly monitoring routine and alert system would trigger the production operators to take immediate action (“biofloc treatment”).

Despite the lack of statistical data on shellfish-borne disease there are indications that bacteria and viruses from the human/animal reservoir are the most common pathogens of concern in both wild-caught and cultured shellfish. The detection of Salmonella and coagulase-positive *Staphylococcus* in shrimps is according to previous studies rare, and the eventual presence in the final product placed on the market primary indicates inadequate hygiene and sanitation routines (PRPs) during harvest, processing and transport. Since the major handling steps are post-harvest when the temperatures are supposed to be about 0-3 °C, contamination and growth by these bacteria is unlikely if the GHP practices are sufficient. Theoretically, pathogens from the human/animal reservoir will not grow optimally during the pre-harvest steps and pathogens from the aquatic environment will not grow optimally during the cold post-harvest steps, and neither of them will survive the intended heat treatment prior to consumption. The flow chart of the production is reasonably simple, including the harvest and post-harvest steps. The fact that there is no shucking or shelling procedure involved, the most common pathway for contamination may be disregarded. However, the importance of the hygiene and sanitation practices must be emphasized since the infective dose of some common human/animal reservoir pathogens are as low as a few cells. In the case for *Listeria monocytogenes*, which origin from the general environment, previous studies indicate that contamination mainly occurs during the post-harvest steps. Again, the PRPs play the most important role to avoid contamination, which is why the HACCP plan results in only one CCP (which aims to keep the cold chain). Ultimately, the final product - the chilled shrimp to be frozen by Findus Sverige AB before placed on the market - is not a sterile product, why it is strongly recommended that the customer information involves requirements of heat treatment prior to consumption.

6.3 Remaining questions

As mentioned above, the European Commission have no established limits for the content of potentially pathogenic bacteria in frozen shrimps to be cooked before consumption that would render the product unfit for human consumption. Given that some of the pathogens mentioned as potential significant hazards are common in the aquatic or general environment and that the harvest procedure is labour intensive, VegaFish would gain from establishing own critical limits for pathogens of relevance as one of the final steps in the production line. Another recommended approach would be to extend the monitoring procedure to detect presence of *Staphylococcus aureus*, *Listeria monocytogenes* along with *Vibrio* spp. and members of the *Enterobacteriaceae* family. Furthermore, extensive training of the PRPs in regard of GHP and sanitation standards is needed along with a common understanding among all personnel on why the harvest includes a CCP and how to avoid its incidence.

The addition of extra probiotic bacteria aiming to inhibit growth of pathogens within the biofloc seems to be a common disease management strategy in BFT systems. The knowledge and number of studies regarding this strategy today is, unfortunately, rather limited. A deeper understanding concerning the relation between biofloc microorganisms and pathogens is therefore desirable.

The BFT has a great potential in becoming a future conventional method for supplying people with food fish, but presence of human pathogens and the potential for their growth within the biofloc needs further examination. However, VegaFish will, with PRPs, HACCP and WQM in routine, have an advantage in the effort of attaining food safety compared to other aquacultural productions.

7 Conclusions

Aquacultured shellfish are generally classified as low risk food. Nevertheless, it is of great relevance to understand potential public health risks associated with a shrimp production to be able to manage them in an effective way. For VegaFish's part, a combination of PRPs, HACCP, water quality management and extensive training is likely to encompass and prevent the major risk of food-borne disease outbreaks. A well-managed, "healthy" biofloc and careful handling during harvest and postharvest procedures are the main preventive measures to avoid contamination by bacterial pathogens. The nature of the biofloc concept, the weekly monitoring program and the low temperatures during post-harvest procedures are predicted to efficiently decrease the risk of bacterial, viral or algal pathogens getting a foot-hold in the shrimps.

The HACCP is a living document and is supposed to be revised at any time when the production is modified, no matter size of the changes. The final product, like any food unless heat sterilized, may contain low numbers of pathogens. Therefore it becomes necessary to include information about the requirement of proper cooking before the product is fit for human consumption in the product declaration towards consumers.

Acknowledgements

I would like to thank my supervisor Matilda Olstorpe, founder and CEO of VegaFish, for letting me write this thesis for her organisation. It has been very stimulating and challenging as I have been able to take part of this innovative company at the very start and building of a food production which I am sure will play an important role in the food sector now and in the future.

I would also like to thank Anna Andreasson, facility manager of VegaFish in Bjuv, for the guidance and discussions concerning my work and all guided tours within the commercial production facility during my visits to Bjuv.

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I also have the luck to have Hans Jonsson as examiner for this thesis. His attempts to remind me about what of what he once taught me about HACCP during the food safety and quality management course a couple of years ago has been quite successful, not rarely by questioning my almost concluded CCPs. It has been instructive and I am very grateful for this.

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Unpublicated material

Åsa Rosengren, Livsmedelsverket. 2015-04-21 (phonecall)

Appendix 1. Implementation of the HACCP principles

The content of this HACCP guide is based on the *Guidance document on the implementation of procedures based on the HACCP principles, and on the facilitation of the implementation of the HACCP principles in certain food businesses* (EC, 2005b), if not other sources or references are given.

Risk assessment

1. **Assemble a multidisciplinary HACCP team.** This team should involve all parts of the food business operator, with the primary goal to develop the HACCP plan.
2. **Describe the product.** A full description is recommended, including relevant safety aspects such as composition, structure and physic-chemical characteristics, processing, packaging, storage and distribution conditions, shelf life, instructions for use/further processing and potential microbiological or chemical criteria.
3. **Identify the intended use.** Define the normal or expected use of the product, and the customer/consumer target group(s).
4. **Construct a flow chart.** Describe the manufacturing process by defining all steps in the chain, e.g. preparation, processing, packaging, storage and distribution, from raw materials to final product released on market.
5. **Confirmation of the flow diagram by the HACCP team.**
6. **Identify and list potential hazards and their control measures.** Hazards can be defined as any biological, chemical or physical property of the product which may cause a human health risk if not controlled. List the potential hazards that are fairly expected to occur at a certain step in the flow chart, including qualitative/quantitative evaluation, unacceptable limits for their presence and possible contamination sources. Also, consider control measures (preventative/eliminating/reducing) which can be applied for each hazard. Sometimes more than one control measure is required for controlling one hazard.

Definitions of Critical Control Points, CCPs

According to Kanduri & Eckhardt (2002), *"a CCP is defined as a point, step or procedure where a food safety hazard can be prevented, eliminated or reduced"*. A CCP may be regarded as a point where an error can cause an instantaneous change. The determination of a CCP requires a logical approach, which can be facilitated by using a "decision tree", see figure 6. The decision tree is built upon four questions that will help the HACCP team determine the CCPs. Examples of a

CCP could be chilling, cooking, prevention of cross-contamination and environmental sanitation.

Establish critical limits

Each CCP needs a specification for its critical limits, i.e. the extreme values acceptable to keep the product safe. Critical limits separate acceptable from unacceptable by observable or measurable parameters such as pH, time, temperature, water activity or sensory values (e.g. visual appearance or texture). The validity of the measurements is of great importance.

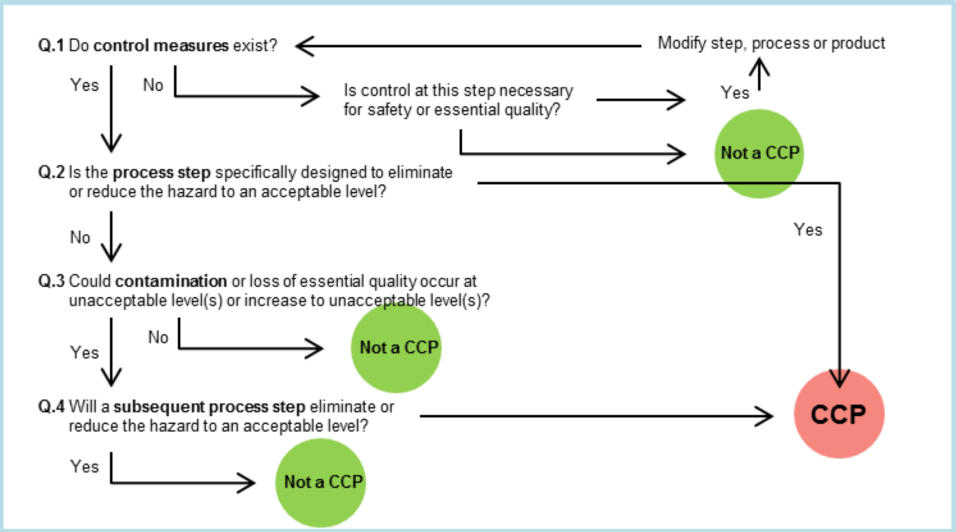


Figure 6. Example of a decision tree used to identify CCPs, based on the code of practice by Codex Alimentarius (CAC/RCP 52-2003)

Monitoring procedures of the CCPs

Monitoring is a program of measurement and observations needed to control each CCP. They need to be able to detect loss of control at critical control points and also give sufficient data needed for corrective action (see next section). The monitoring program should be defined for the following questions:

- What will be measured/observed?
- How will the measurements/observations be done?
- What is the required frequency of the monitoring?
- Who is to perform the measurements/observations? The person(s) performing the test need to be trained and understand the purpose and the importance of the monitoring (Kanduri & Eckhardt 2002).

Set corrective action to implement when a CCP is monitored

Corrective actions have to be defined when developing the HACCP plan so that they can be performed immediately when the monitoring results is indicating that a CCP is deviating towards its critical limit. When planning the corrective actions one should include (i) who is responsible for implementing the corrective action, (ii) description of the corrective action, i.e. description of what needs to be done to correct the observed deviation, (iii) what to do with the food that have been produced during the time when the CCP is out of control (depends on the monitoring frequency), and (iv) documentation of the deviation and the corrective action.

Establish a HACCP verification procedure

In order to ensure that the HACCP plan is working correctly, some procedures of verification is needed. The verification may include random sampling and analysis of CCPs to confirm that they are kept under control, validation of critical limits or inspection of operations within the program. If possible, the verification procedure should confirm that all element of HACCP is working. If any change is done within the flow chart the system might need a rearrangement.

Establish procedures for record keeping and documentation

Documentation and record keeping is essential to the application of HACCP. The documentation should include:

- Hazard analysis
- CCP determination
- Determination of critical limit(s)
- Modifications of the HACCP plan, when needed

Records should include:

- CCP measurements and observations
- CCP deviations and their corrective actions
- Verifications

Training

The food business operator has responsibility to ensure that all employees are aware of the identified hazards (if any) for every specific product. This includes information about the CCPs, corrective actions and the documentation process in his/her certain part of the manufacturing, and that the employees get sufficient training when needed.

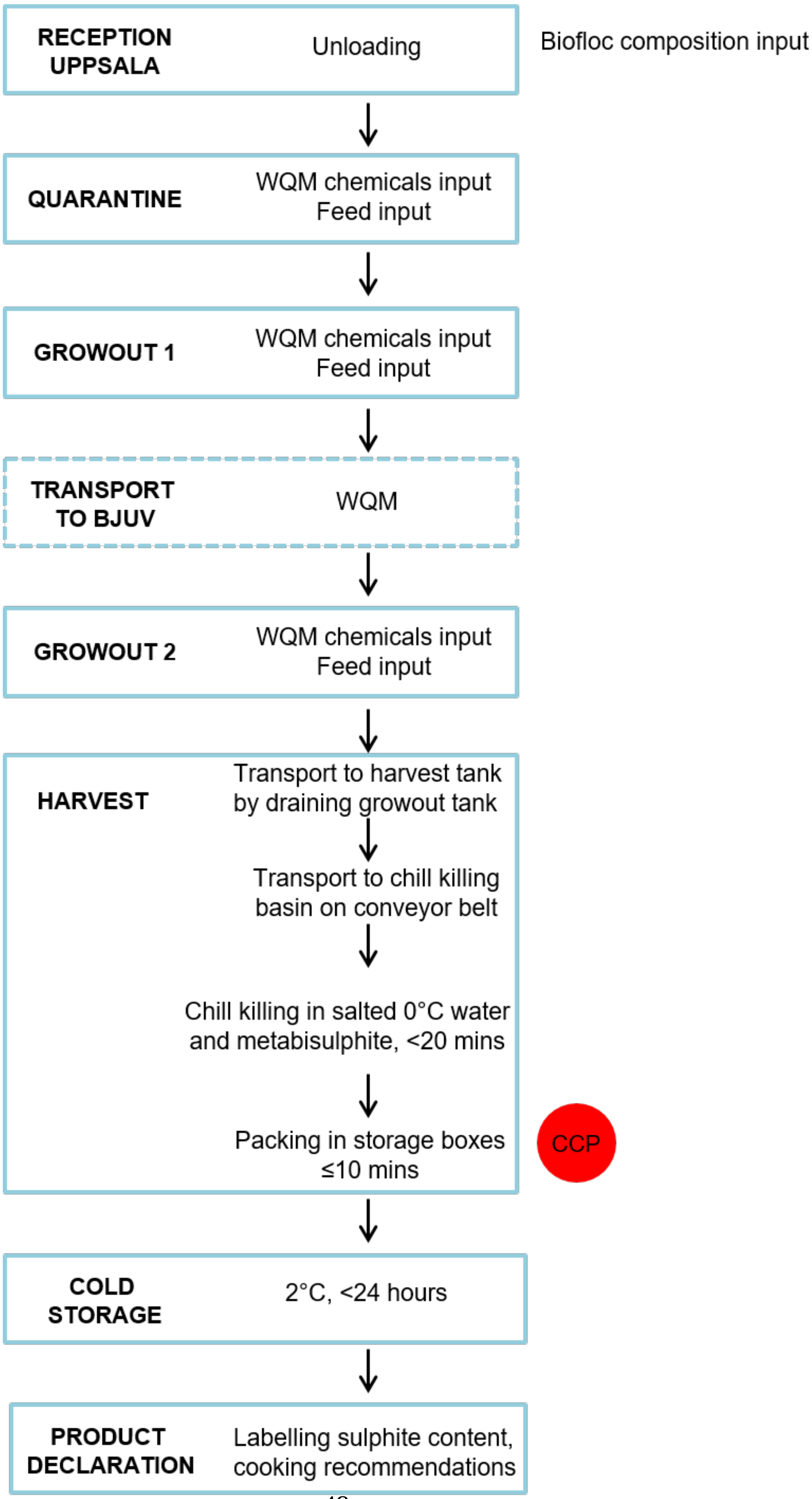
Appendix 2. Listed Specific Pathogens (LSP)

The table 9 below lists pathogens associated with shrimp aquaculture and certain steps/points during the production prone to contamination are presented.

Table 9. *Listed specific pathogens concerning aquaculture production of shrimp.*

Primary production step/source of contamination	**Origin of pathogens**	**Pathogens of primary concern**	**References**
Facilities and equipment	Human/animal reservoir	*Campylobacter jejuni, Staphylococcus aureus, E. coli, Listeria monocytogenes*	Huss *et al.* (2004) Norhana *et al.* (2010)
Reception - Shrimp larvae and/or water	Aquatic environment	*Vibrio* spp. especially *parahaemolyticus, vulnificus* and *cholerae*	Brito *et al.* (2015) Yingkajorn *et al.* (2014) Huss *et al.* (2004)
	Human/animal reservoir	Coliforms, e.g. *E.coli* Salmonella	Brito *et al.* (2015) Amagliani *et al.* (2012) Norhana *et al.* (2010)
Biofloc preparation - Microbiological components	Aquatic environment	*Clostridium botulinum* type E, Plesiomonas shigelloides, *Aeromonas* spp.	Liu *et al.* (2015) Huss *et al.* (2004) Huss (2000) Reilly & Käferstein (1997)
	General environment	*Listeria monocytogenes, Pseudomonas* spp., *Clostridium botulinum* type A, B, *Bacillus*, algal toxins	Huss *et al.* (2004)
Formulated shrimp feed /fertilizers	Human/animal reservoir	Salmonella, *Shigella* spp.	Norhana *et al.* (2010) Brito *et al.* (2015)
Harvest – Personnel handling	Human/animal reservoir	Salmonella, *Staphylococcus aureus, E. coli,* Hepatit A virus, Rotavirus, Norovirus, Calicivirus	Huss *et al.* (2004) Amagliani *et al.* (2012) Brito *et al.* (2015) Jones (2009)
	General environment	*Listeria monocytogenes, Pseudomonas* spp., *Clostridium botulinum* type A, B	Huss *et al.* (2004) Norhana *et al.* (2010)

Appendix 3. Flow chart



Appendix 4. Popular summary

Shrimps produced in a microbial co-culture – will they be edible?

Shrimps, prawns, scampi or gambas. Culinary delicacies to some people, disastrous environment desolaters to others. Both, to some. What about more ecologically sound shrimps produced in land-based basins, spending their days in a small ecosystem eating co-cultures of bacteria and algae, would they be safe to eat? Are we facing the next generation in aquaculture?

In this study, a step by step risk analysis have been made to evaluate the food safety of white-legged shrimps produced by the company VegaFish AB. The production is held in indoor basins in Sweden, a country far away from Thailand and the U.S.A where they are traditionally produced in warm estuarine waters and ponds. The system is based on the Biofloc Technology method (BFT), a system for closed productions, where an ecosystem of friendly bacteria, algae and zooplankton feeds the shrimp population.

Although shrimp tend to spoil rapidly after harvest the worldwide compliance with food safety standards and regulations are somewhat defective. This study is based on current food standards and concludes that the VegaFish shrimps will be safe to consume. In simple terms, there are two actions which need to be taken to assure the food safety of these shrimps. First, the producer has to make sure that the shrimps are kept in cold storage within 30 minutes after harvest to avoid spoilage. Second, the consumer has to make sure that the shrimps are properly cooked before consumption. If these two actions are taken, the probability of public health disease outbreaks is minimised.

The demand for shrimps are increasing on a global scale, but the increased production causes destruction of whole ecosystems, depletion of fresh water resources and land seizure. The mangrove removal has gotten as much attention as the devastating situation for local inhabitants, which may get stuck in their new, depleted, environment far after the global aquaculture company has left the place. The aquaculture sector needs to face a transition towards more ecologically sound production methods. Therefore, systems like the BFT method might be the next generation in aquaculture. This study, containing a thorough step by step risk analysis might be of relevance in future developments within the aquacultural sector.