UiT The Arctic University of Norway

Institute of Arctic Marine Biology

From Trash to Treasure: The use of Lumpfish (*Cyclopterus lumpus)* as feed for Red King Crab (*Paralithodes camtschaticus*).

Exploring the potential of upcycling aquaculture by-products to enhance sustainability and economic viability by feeding juvenile red king crab with lumpfish.

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Illustrated by Oddvar Dahl, Nofima.

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Abstract

This thesis explores the potential of using farmed lumpfish (*Cyclopterus lumpus*), discarded from Atlantic salmon (*Salmo salar* L.*)* aquaculture industry, as a sustainable feed for juvenile red king crabs (*Paralithodes camtschaticus*). The red king crab has successfully established a self-sustaining population in the Barents Sea. With the red king crab being a large generalist predator, its impact on native bottom communities is a concern. Additionally, the red king crab fishery is a valuable resource, while the lumpfish is an important natural alternative for treating sea lice (*Lepeophtheirus salmonis)* in salmon aquaculture. The lumpfish stops eating lice when reaching sexual maturity, the fish is then discarded. The study involved feeding four different diets to juvenile red king crabs over 12 weeks, consisting of dry feed, dry feed coated with lumpfish hydrolysate, a combination of dry feed and fresh frozen lumpfish and fresh frozen lumpfish. The crabs receiving mixed diets was seen to have the highest feed intake. The results suggest that lumpfish can be used as feed, providing a basis for better utilization of the resource while reducing pressure on the ecosystem by removing juvenile king crabs from the sea. This approach would enable the harvesting of one resource, the utilization of a "by-product", and a reduction in negative impacts on the ecosystem. Ultimately, this research promotes sustainability and responsible resource management.

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1. Introduction

1.1 King Crabs and the Red King Crab (*Paralithodes camtschaticus,* Tilesius 1815)

The King crab, a species of crustacean, is renowned for its remarkable size and commercial value (Lorentzen et al., 2018). This species is classified under the phylum Arthropoda, order Decapoda, and suborder Eucarida. The crab's rigid exoskeleton, composed mostly of chitin, covers all external body parts. The carapace is the structure that covers the cephalothorax which is the fused head and thorax (Donaldson & Byersdorfer, 2005).

The red king crab (RKC) is a widely recognized species with various common names, such as the Kamchatka crab or the Alaskan king crab (Dew & McConnaughey, 2005; Galil et al., 2011). The RKC is believed to have evolved from the asymmetrical long-tailed hermit crab through the squat lobster, ultimately taking on a crab-like morphology (Tsang et al., 2011). This transformation involved broadening the cephalothorax and shortening the pleon, which is now tucked and folded under the cephalothorax (Tsang et al., 2011). The RKC is the largest of the king crab species, with some individuals exhibiting carapace lengths and widths of up to 20 cm. Adult males can grow to a maximum length of 1.8 meters and weigh up to 11 kg. Larger specimens have been recorded but are rare. It is worth noting that the average size of RKC caught in fisheries is around 3 kg (Stevens, 2014) and has a lifespan of up to 20–30 years (Kovatcheva et al., 2006). The RKC has three pairs of walking legs, one pair of chelipeds (legs bearing a claw), and one pair of walking legs that are reduced in size and folded within their gill chambers. The chelipeds are unequal in size, with the right used as a crusher and the left as a cutter. The entire body of the RKC is covered in sharp spines (Stevens, 2014). The RKC can be found in different temperatures and is a boreal species. They have been found to tolerate temperatures from −1.6 to +18 °C, but thrive in temperatures between 2–7 °C. (Falk-Petersen et al., 2011). The RKC, like other crustaceans, is a poikilothermic organism whose internal body temperature fluctuates with changes in the external environment. Therefore, the temperature of the surrounding environment direct impacts the rate of biological processes, including metabolism, growth, moulting, feeding, and development (Kovatcheva et al., 2006). Different life history stages of the RKC require distinct temperature ranges for optimal performance. Due to their high temperature tolerance, the RKC populations are not restricted to the north and may spread into southern regions of the Norwegian coast.

1.1.1 Moulting

Moulting is a crucial process for king crabs' growth and survival. Adequate nutrition and the right temperature are essential to facilitate the development of a new exoskeleton (Nilssen & Sundet, 2006). In king crabs and other crustaceans, the hepatopancreas is a vital organ responsible for nutrient metabolism and energy production. The energy generated by this organ

plays a crucial role in various physiological processes, including moulting, energy homeostasis regulation, and post–moulting survival (Wang et al., 2014). During the process of moulting, also known as ecdysis, it is common for crabs to significantly reduce or even cease their food intake as they develop a new exoskeleton within their original exoskeleton. Once the new exoskeleton is ready, the crab will shed its old and emerge with a soft and pliable one, which gradually hardens over time. The crab can be anywhere from 15–40 % bigger than before. About 1–2 months before moulting, an extra inner membrane is created between the muscle and exoskeleton, known as a "double shell" (Stevens, 2014). Prior to moulting, the king crab initiates a process of water absorption which occurs within a time frame of 24–48 hours, leading to visible abdominal distension in the crustacean (Stevens, 2014). Following the ecdysis, a stage known as post–moult commences, during which the crab absorbs water and expands in size while still retaining its vulnerable and soft exoskeleton. This stage involves the continued absorption of water until the new exoskeleton has fully hardened (Luppi et al., 2004). Once the new exoskeleton has hardened, the crab can resume normal activities, such as hunting and reproducing.

Calcium is a crucial component for the hardening of the crab's exoskeleton. Typically, this mineral is the most abundant cation in the crab's body, and during intermoult, the crab is in a state of calcium equilibrium with its environment (Greenavvay, 1985; Jørgensen et al., 2005). However, calcium is typically lost to the environment during premoult, and the storage becomes depleted (Philips, 2000). Recalcification is necessary for the hardening of the exoskeleton and commences immediately following ecdysis. Due to the crab's low calcium storage, it is essential to replenish the mineral through diet and water absorption (Greenavvay, 1985; Jewett & Feder, 1982; Kovatcheva et al., 2006). During the moulting process in crabs, the muscle is reduced in size compared to the newly formed exoskeleton, and the void space in the exoskeleton is occupied by water (Kovatcheva et al., 2006).

Consequently, the weight of the crab after ecdysis and right before the following pre–moult period remains comparable due to the water content being replaced by the growth of muscle (Stevens, 2014). Additionally, the moulting process facilitates wound healing and the removal of undesired organisms growing on the exoskeleton. Overall, moulting is a unique growth style found only in Arthropoda, and it plays a critical role in the life cycle of King crabs. Adequate nutrition and proper care during moulting are essential for the crab's growth and survival. Nonetheless, only a few systematic studies on the nutritional requirements of the RKC have been conducted. The reproductive process of king crabs is intrinsically linked to their moulting behaviour. Specifically, female specimens undergo moulting prior to spawning, while male individuals exhibit a more erratic moulting pattern. The frequency of moulting in juveniles is notably higher, with up to six occurrences per year, compared to 1–2 moults annually in post-juvenile stages (Stevens, 2014). Fully grown male king crabs usually moult once a year, though it has been observed that mature males do not exhibit annual moulting behaviour (Nilssen & Sundet, 2006). Notably, the primary moulting season for males inhabiting the Bering and southern Barents Sea occurs between January to April (Nilssen & Sundet, 2006).

1.1.2 Behavioural ecology

During the first year of life, RKC juveniles are typically solitary but begin to form stacks or "pods" during their second year (Dew, 1990). This podding behaviour occurs in shallow water and is typically limited to juvenile crabs until their fourth year of life (Galil et al., 2011). The podding serves as protection from predators as well as increased opportunities for feeding (Cárdenas et al., 2007). Mature RKCs are typically found on soft mud bottoms at greater depths and often associate with conspecifics of similar size and sex (Pedersen et al., 2006). Around their fourth year, the juvenile crabs will join the mature crabs in the deeper waters for the moult and mating migrations (Kovatcheva et al., 2006). The female RKCs usually mature when 6–7 years old with a carapace of about 8–9 cm. However, the male RKCs are usually 7–10 years old before reaching maturity, with a carapace width of 12.5–13.0 cm (Kovatcheva et al., 2006). Like other crustacean species, the RKC establishes behavioural hierarchies within its pods (Phillips, 2008). Such hierarchies are based on the size and age of individuals, with larger and older ones having greater access to available food resources and the ability to influence the growth trajectory of smaller individuals. It is noteworthy that the formation of new pods or changes in group composition may result in alterations to the established hierarchical structures (Phillips, 2008). Cannibalism has been observed both in laboratory experiments and in the wild (Long et al., 2012). This phenomenon is typically observed among RKC of varying sizes and ages (Stevens & Swiney, 2005).

1.1.3 Feeding ecology and prey diversity

The RKC is known to be a generalist predator and omnivorous in nature (Britayev et al., 2010). Although characterised by predatory behaviour, this species will also scavenge on available food resources (Falk-Petersen, 2014). The dietary preferences of the RKC vary throughout its lifespan. During its larval stage, the RKC feeds on phytoplankton and zooplankton, underscoring the importance of matching hatching and drifting with blooms (Galil et al., 2011; Pedersen et al., 2006). After settling, the RKC's diet primarily consists of hydroids. As the RKC grows, the diet diversifies to include barnacles, macroalgae, egg clutches, sea stars, and bivalves (Galil et al., 2011). Juvenile RKCs have high prey diversity and have been observed to consume up to 100 different species, with bivalves, molluscs, and polychaetes dominating their diet (Britayev et al., 2010). Upon reaching adulthood, RKCs are considered opportunistic and generalist feeders, consuming various food sources across different habitats (Fuhrmann, 2016), including benthic fauna in deep soft-bottomed areas (Oug et al., 2018). Both juvenile and grown RKCs feed on multiple trophic levels, with a top-down role in the ecosystem (Fuhrmann, 2016). Studies show that juvenile RKCs have a higher food intake compared to weight than adult RKCs (Stevens, 2012). The difference in the amount eaten between RKCs under 140 mm and over 140 mm can be up to 40% (Jewett & Feder, 1982).

The RKC employs its chelipeds to grasp and tear food items and may engage in excessive predation, leading to partial consumption of prey (Jørgensen et al., 2005; Kovatcheva et al., 2006; Pedersen et al., 2018). In addition to using the chelipeds when grasping, the RKC uses

the third maxillipeds and the lesser chela to sieve out organisms occupying the sediment (Jørgensen et al., 2005). Smaller crabs have been shown to damage more prey without eating them than larger crabs; the spillage declines with increased size (Britayev et al., 2010). Crustacean studies have revealed that the proventriculus of these organisms is small and thus fills quickly, providing insight into why crustaceans require frequent, smaller meals (Lee & Lawrence, 1997). The proventriculus is a part of the digestive tract that includes a relatively short oesophagus leading to a stomach. This stomach, also known as the foregut, can be divided into an anterior (cardiac) and a posterior (pyloric) section (Watling, 2013). Furthermore, investigations have shown that 75% of ingested food is cleared from the foregut within one hour, indicating that crustaceans can regularly ingest small portions (Lee & Lawrence, 1997).

1.1.4 Resource, introduction, and distribution

The native range of RKC includes the North Pacific, Bering Sea, and Okhotsk Sea, while a fourth population of RKC has been introduced in the Barents Sea north of Murmansk (Stevens, 2014). The introduction was carried out with the explicit objective of establishing a sustainable commercial fishery and took place between 1961 and 1969 (Galil et al., 2011). Following its intentional introduction, the species has successfully established a self-sustaining population that has steadily expanded its geographical distribution. The RKC has spread southwards along the Norwegian coast. However, since 1990, the population of RKC in the Barents Sea has increased, leading to the species being classified as blacklisted (Falk-Petersen, 2014). The RKC was first introduced into the Kolafjord, situated in the eastern part of the Barents Sea (Oug et al., 2018). The species exhibited the highest population densities in the late 1980s to the early 1990s, primarily in the Russian sector of the Barents Sea. During the 1990s, the RKC was detected along the stretch from Cape Kanin to the White Sea, with observations extending until 2002 (Galil et al., 2011). Subsequently, the species abundance was found to be more significant in Norwegian waters in the early 1990s, after which it progressively migrated down the Norwegian coastline over the following decade, see Figure 1 (Falk-Petersen et al., 2011). With the current knowledge of migration rate, it has been estimated that RKC can be established around Trondheim by 2050[1].

[1] Citation Carsten Hvingel, Head of Research at Norwegian Institute of Marine Research (IMR) (*Havforskningsinstituttet*), newsletter November 2019.

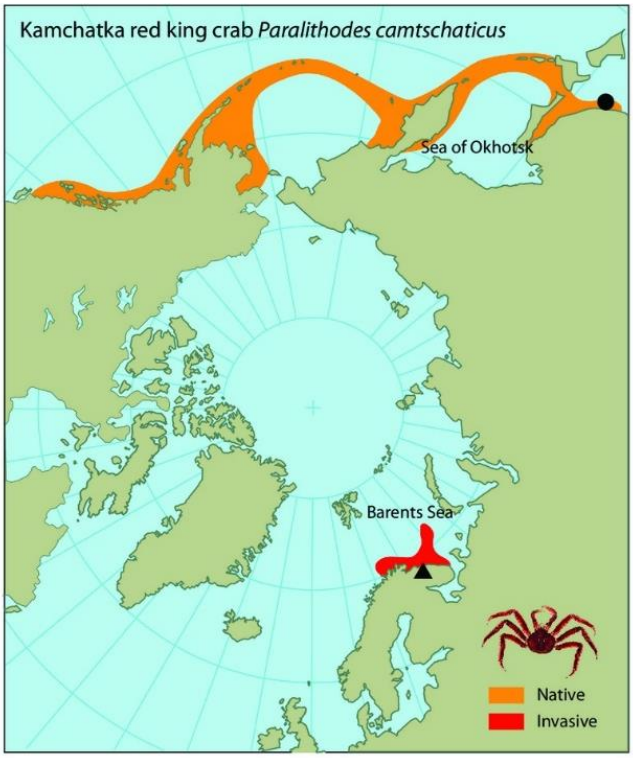

Figure 1. Distribution of RKC. Orange areas indicate where RKC is native, whereas red areas indicate where RKC is invasive (Christiansen et al., 2015).

The first documented capture of the RKC in Norway was in 1977 (Sundet & Hoel, 2016), with commercial fishing operations commencing in 2002 (Oug et al., 2018). However, even prior to commercial fishing, the RKC had already caused problems for local fisheries through incidental capture. These crabs damaged fishing equipment and interfered with catching the targeted species (Galil et al., 2011). To manage the commercial fishery of RKC, the 3-S regime, which accounts for sex, size, and season, is employed in the relevant area (Nilssen & Sundet, 2006).

Fishing during the moulting period or shortly after that is not recommended due to the potential harm to the RKC from fishing equipment and the low meat content of the crab, rendering it unsuitable for sale (Stevens, 2009). Thus, it is essential to determine the RKC moulting season for appropriate fishing times, which usually occur between November and April (Nilssen & Sundet, 2006). The males in the Bering Sea usually moult between January and April, and the females a few weeks later (Nilssen & Sundet, 2006). However, fixed set fishing seasons may pose challenges due to variations in moulting times, especially if the season is cold and RKC moult later than in previous years. Therefore, knowing the moulting schedule is critical to ensure the crab has enough time to harden its exoskeleton post-moult and accumulate sufficient meat content to meet market demands. Moreover, only male RKC are harvested in commercial fisheries, and fishing during mating and moulting periods is prohibited (Stevens, 2009). Since 2008, the RKC fishery in Norway have had two main objectives (*Stortinget*, 2020), namely, to maintain an economically viable fishery within a geographically limited area while preventing further spread outside the set area. The Norwegian RKC fishery is quota-regulated east of 26th meridian east whereas a free fishing area (FFA) is set west of this meridian. The FFA serve as an eradication fishery and hinder the spread of the RKC further down the coast. The government

aims to establish a large reproducing population in the regulated are to support commercial fisheries (Windsland et al., 2014). Despite the FFA, the RKC distribution range continuously expands along the Norwegian coastline, with established populations observed in Balsfjorden (Fuhrmann, 2016). The minimum legal landing size in the regulated area is 132 mm carapace length or 150 mm carapace width (Sundet & Hjelset, 2002). In the FFA, all RKCs are to be landed. It is estimated that it is five to eight times more juvenile RKC than RKCs of commercial size (Gudimov et al., 2003). A grant program was implemented in 2010 for RKC fishing west of 26°E to encourage greater catch effort and restrict the spread of the species. The provision of grants to vessels ensures that all caught crabs are landed, regardless of size or quality (Fiskeridirektoratet, 2014). According to a landing facility in Honningsvåg, that has utilized the grant program, vessels that deliver RKC from open fishing grounds generally refrain from landing small crabs outside the grant period. This observation suggests that small crabs are sorted out at sea and not landed during non-grant periods. Companies have indicated that they require grants to manage landed crabs that are unsuitable for further processing or live sales, particularly juvenile RKC. Without the grants, they are unwilling to accept small crabs due to financial considerations (Fiskeridepartementet, 2015). The grant program was terminated in 2019, and the initiative to land juvenile RKC was dismissed (G. Lorentzen, pers. comm).

Typically, RKCs are caught in the intertidal region to the continental slope, with depths ranging from 9–460 meters (Stevens, 2014). In Norwegian waters, they can be found in fjords and along the coast throughout the year due to the steeper topography of the Norwegian waters (Galil et al., 2011). The RKC fishery represents a valuable resource, with Norway exporting 2261 tons of RKC valued at 999 million Norwegian kroner in 2021 (Norges Sjømatråd, 2022). According to Nilsen et al. (2019), a total of 147 tonnes of juvenile RKC were landed in the FFA zone between 2012 and 2018. However, due to the lack of incentives for fishers to land juvenile RKC and the return of these juveniles to the ocean (S. Siikavuopio, pers. comm). Together with the large net size used in traps that may cause juveniles to evade capture (Stevens, 2014), it is likely that these numbers underestimate the true extent of juvenile RKC in the area.

1.1.5 Ecosystem effects

In contemporary times, biological invasions have become increasingly pronounced due to intensified human activity and rising temperatures (Williamson, 1996). Biological invasion refers to the spread of a species into an area previously outside its distribution range, with human actions, whether intentional or accidental, often responsible for such invasions. The impact of invasive species on natural ecosystems is multifaceted, ranging from alterations in the habitat and ecosystem to interactions with local fauna (Williamson, 1996). It is worth noting that only about 10% of introduced species succeed in establishing a reproductive population; however, once established, they can become difficult to eradicate. As the incidence of invasions continues to increase, local ecosystems may be significantly affected and altered (Oug et al., 2011). Specifically, the RKC, as a large generalist species, is expected to exert a considerable influence on the benthic fauna of the region (Marukawa, 1933).

The successful establishment and proliferation of the RKC in the Barents Sea can be attributed to several factors. Firstly, upon its introduction to the region, there was no fishing on the species as Russia initially claimed ownership of the stocks, which delayed the start of fishing until 2002, when research fishery began (Broderstad & Eythórsson, 2014). This allowed the RKC to establish a reproducing population without significant anthropogenic pressure. Additionally, the RKC possesses several biological traits that contribute to its success in an unfamiliar environment, such as its long lifespan, slow growth, and high reproductive potential (Windsland et al., 2014). Moreover, its opportunistic and generalist feeding behaviour enables it to adapt and thrive in new environments (Fuhrmann, 2016). The RKC also displays a remarkable ability to endure variable environmental conditions, including variations in salinity and temperature (Jørgensen et al., 2005; Windsland et al., 2014).

Research showed that the RKC might disturb benthic communities and alter the diversity and composition of ecosystems, with a decline in abundance of species with low mobility, such as echinoderms, molluscs, and borrowing species, and an increase in abundance of more mobile species that are able to escape the RKC (Anisimova et al., 2005; Gerasimova, 1997; Oug et al., 2011). RCKs have been observed to restructure the physical habitat and to function as ecosystem engineers (Falk-Petersen et al., 2011). The RKC forages the sediment by moving it with its third maxillipeds. This may result in deeper sediment layers having less oxygen due to the RKC foraging on borrowing species, which moves oxygen down into the sediment. This could affect benthic-pelagic coupling by altering energy flow in the waters and, in turn, affecting pelagic and benthic life (Fuhrmann, 2016). Competition between the RKC and fish stocks has been observed indirectly in the Barents Sea, with the RKC consuming fish eggs from caplin and lumpfish. In addition, RCKs feed on kelp that serves as hiding spots for juvenile fish. It is noteworthy that sea urchins have been observed to consume more kelp than the RKC. In the Porsanger fjord, the RKC may positively impact kelp populations by preying on sea urchins (Fuhrmann, 2016; Gudimov et al., 2003). While the RKC may compete with fish for food resources, studies have shown that RCKs occupy a unique niche in the food web that does not overlap significantly with fish (Fuhrmann, 2016). The RKC has been shown to have an impact on benthic populations due to competition with big benthic species, but not significantly affect pelagic populations (Falk-Petersen, 2004) (Fuhrmann et al., 2017). The distribution of the snow crab (*Chionoecetes opilio*) and the edible crab (*Cancer pagurus*) overlaps with that of the RKC. The high foraging behaviour of these three species may affect the ecosystem, and competition between the three species may also be observed (Bakke, 2019; Lorentzen et al., 2018).

1.2 The biology and aquaculture significance of the lumpfish (*Cyclopterus lumpus. L*)

The lumpfish (*Cyclopterus lumpus*) is a marine fish species belonging to the family Cyclopteridae (Powell, Pooley, et al., 2018). The species has a distinctive appearance, with a compressed body and a sucker disk on its ventral side, which is used to attach to substrates (Davenport, 1985). Lumpfish are benthic feeders and primarily consume large planktonic organisms, benthic organisms, and some copepods (Davenport, 1985). They are distributed in the Barents Sea, Atlantic Ocean, North Sea, and Baltic Sea (Cox & Anderson, 1922; Davenport,

1985). Lumpfish are being farmed due to their importance in the Atlantic salmon (*Salmo salar* L.*)* aquaculture industry as a natural alternative to chemical treatments for sea lice (*Lepeophtheirus salmonis)*. Sea lice cause a parasitic infection that leads to economic losses in Atlantic salmon farming (Powell, Pooley, et al., 2018; Powell, Treasurer, et al., 2018). Farmed lumpfish are stocked together with salmon in the pens to feed on sea lice that attach to the skin of the salmon (Jónsdóttir et al., 2022). The use of lumpfish as a cleaner fish in the salmon industry has become essential in many Norwegian salmon farms, with over 42 million lumpfish juveniles being used for this purpose in 2019 (Ageeva et al., 2021). However, the welfare of lumpfish in aquaculture is still a matter of concern (Garcia de Leaniz et al., 2022). The pens are designed for the salmon and not for the lumpfish. Moreover, lice are insufficient feed sources for the lumpfish (Garcia de Leaniz et al., 2022). Once the lumpfish reach sexual maturity, they stop eating lice. At this point, the fish is usually taken out of the pens and either discarded, used as low-value silage, or used in ensilage (Nøstvold et al., 2016). The lumpfish from aquaculture is deemed safe to eat, but due to small size and the lack of interest, it is mainly disposed of (Ageeva et al., 2021).

1.3 Research objectives

Scarcity of resources, including food, is an increasing global concern, often coupled with poor resource management practices. This presents a pressing need for sustainable resource utilization to ensure their availability for future generations. The RKC represents a significant resource; however, as a blacklisted invasive species in the Barents Sea, its spread and disruption of natural ecosystems are a cause for concern (Gederaas et al., 2012; Sundt et al., 2012). To address this issue, there is a need to prioritize ways of maintaining RKC as a resource while limiting its spread. For this reason, the Norwegian RKC fishery is quota-regulated. In the FFA, juvenile RKCs are observed to be returned to the ocean to preserve the resource for future years, but this contributes to the spread of the species (S. Siikavuopio, pers. comm). As well as returning the juvenile RKCs to the ocean, the mesh size in the traps is large, causing the juvenile RKCs to avoid being caught (Stevens, 2014). Initiatives encouraging fishers to bring the juvenile RKCs to shore for commercial purposes could help hinder the spread of the species. In this thesis, farmed lumpfish, a residual raw material from the salmon aquaculture industry is used to feed juvenile RKCs. Utilizing lumpfish as a feed source for RKC could create a win-win situations for all stakeholders and the ecosystems. The thesis focuses on assessing the adequacy of farmed lumpfish as a feed source for RKC, with the aim of boosting their appetite and reducing their reliance on dry feed. It is expected that dry feed coated with lumpfish hydrolysate will have an increased attractiveness compared to standard feed due to the presence of water-soluble peptides from lumpfish (Stevens, 2014). The optimal feeding strategy should involve better usage of lumpfish supplemented with dry feed to meet the nutritional requirements of the RKC. This thesis is part of the Nofima project "Kongemat" funded by the MABIT program. The three diets found to be most effective in the thesis will be used to feed 300 crabs in a pilot–scale trial in collaboration with Cape Fish Group AS in Honningsvåg.

2 Materials and methods

2.1 Raw material

Juvenile male RKCs, weighing between 334 and 1052 grams, were collected in Alta fjord in May 2022 and transported in dry storage to the Tromsø Aquaculture Research Station (ARS) in Kårvik. The dry feed utilized in the study was produced by the Aquafeed Technology Center (ATC) at Nofima in Bergen, based on a formulation developed by Nofima (Siikavuopio & James, 2015). The specimens of lumpfish used in the study were collected from fish farms operated by SalMar Nord (Senja) as well as from the ARS. As the lumpfish was intended for consumption, they were manually slaughtered without the use of anaesthesia. The head and fins were subsequently removed due to observations indicating the RKC's inability to consume them. The rest of the fish was cut into approximately 1 cm^3 cubes and frozen (Figure 2).

Figure 2. The lumpfish was cut into cubes of approx. 1 cm^3 before being fed to the RKCs. Photo: Tora M. Conradi-Larsen.

2.1.1 Enzymatic hydrolysis of lumpfish

Ground lumpfish was mixed with distilled water at a 1:1 ratio in an LR 1000 basic system High Viscosity Reactor (IKA, Steufen, Germany). Upon reaching 65 °C, the mixture was added proteolytic enzyme 1% Corolase8000® (Novozymes) per gram of raw material. The blend was stirred for one hour at 50 rpm before the temperature was increased to 95 °C and held for 15 min to inactivate the enzyme. After cooling, the mixture was passed through a sieve to remove bones and undissolved protein. The remaining water phase (hydrolysate) was filtrated with a grade 4 320 mm filter paper to remove impurities and lipid residues. The hydrolysate was then evaporated to 40º brix using a hei-VAP Industrial rotavapor with glassware R (Heidolph, Schwabach, Germany). The dry feed was coated by mixing 2% lumpfish hydrolysate with RKC dry feed. The feed was subsequently rotated and put under pressure in a Heidolph rotavapor to ensure the hydrolysate was well incorporated into the feed. The feed was stored frozen until the start of the feed study.

2.2 Feed study

Upon arrival at the ARS, 24 RKCs were acclimated to individual chambers (62.5 × 45 × 17 cm) for a period of four weeks, during which they were fed dry feed. The chambers were arranged in groups of four in separate raceways (250 × 45 × 17 cm), with a total of 24 chambers and six raceways (Figure 3).

Figure 3. The set up in the feed study with RCKs in individual chambers at the ARS in Kårvika, Tromsø. Photo: Tora M. Conradi-Larsen.

The weight of the crabs used in the feed study ranged from 334 to 914 grams (Table 5). The RKC was kept solitary so that individual feed intake could be calculated and to avoid competition for feed and cannibalism. Each chamber had a separate water supply to keep replicates completely independent of each other. The water used was natural seawater pumped from a depth of 50 m and 30 m outside of the ARS and filtrated with a particle-filter (60 µm) and a UV filter. The crabs were exposed to a circadian light regime, simulating daily changes according to Tromsø latitude.

After the acclimation period, the feed study started on the 26th of September 2022 and lasted until the 19th of December 2022, during which four distinct diet regimes were established (Table 1).

Table 1. Experimental design with the allocation of RKCs identified by alphanumeric code to diet groups.

Diet group A: Dry feed (*n*=6)	Diet group B: Dry feed coated with lumpfish hydrolysate (*n*=6)	Diet group C: Dry feed and fresh frozen lumpfish (*n*=6)	Diet group D: Fresh frozen lumpfish (*n*=6)
3a	4b	1c	5d
11a	6b	2c	8d
16a	7b	9c	14d
17a	12b	10c	15d
19a	18b	13c	23d
22a	20b	21c	24d

The crabs were fed every Monday, Wednesday, and Friday for 12 weeks, and uneaten feed was removed and weighed every Tuesday and Thursday. Leftover feed and faeces from the week

before were removed but not weighed on Mondays. The dry feed used in the feed study had the same formulation as the dry feed used in the acclimation period except for different binders. Two of the RKCs were not eating during the acclimation period. Therefore, they were replaced with crabs 4b and 22a that had been fed a mix of wet feed, not including lumpfish, during the acclimation period.

2.2.1 Determination of feeding regime

The feeding regime was calculated based on the nutritional requirements estimates of the RKC (D'Abramo et al., 1997) and the nutritional composition of both the dry feed and farmed lumpfish. The information reported by Siikavuopio and James (2015) was used to calculate how much feed was expected to be consumed by each RKC. The detailed nutritional content of the dry feed is given in Appendix Figure S1, while the nutritional value of farmed lumpfish was reported by Ageeva et al. (2021). An overview of the gross nutritional composition of farmed lumpfish and dry feed is given in Table 2.

Table 2. Gross nutritional composition of dry feed and farmed lumpfish produced by Aquafeed Technology Centre (ATC) at Nofima in Bergen. Values are given on wet weight basis.

	Dry feed	Lumpfish
Energy (kJ / 100g)	1999	144
Moisture (%)	10.3	15.9
Fat (%)	17.0	1.3
Protein (%)	50.7	5.7

Equation 1 shows that, on a wet weight basis, an amount of lumpfish approximately 14-fold higher than dry feed was required due to the low nutritional value of the former, as reported in Table 2.

$$Energy\ content\ ratio\ \frac{dry\ feed}{lumpfish}\ \frac{1999kJ/100g}{144kJ/100g} = 13.9 \quad (1)$$

The nutritional requirements of the RKCs were estimated to be 2.5 $g^{-1}\ kg^{-1}\ day^{-1}$ of dry feed and 35 $g^{-1}\ kg^{-1}\ day^{-1}$ of lumpfish, both expressed on a wet weight basis.
It was chosen to provide the RKCs with double the amount of the feed they were expected to consume to ensure that feed availability would not restrict intake and growth. Therefore, the amount of feed for diet groups A and B was set at 5 $g^{-1}\ kg^{-1}\ day^{-1}$. Diet C was composed of a 50 to 50 mixture of 2.5 $g^{-1}\ kg^{-1}\ day^{-1}$ of dry feed and 35 $g^{-1}\ kg^{-1}\ day^{-1}$ of lumpfish, while Diet D was entirely comprised of lumpfish (70 $g^{-1}\ kg^{-1}\ day^{-1}$).

To allow for direct comparisons of feed consumed by each crab, all weights were converted to their corresponding dry matter content. Thus, a correction factor was determined to accurately assess the quantity of feed consumed by each crab, expressed in dry matter. Dry feed, dry feed coated with lumpfish hydrolysate, and lumpfish cubes were placed in a heating cabinet at 95 °C for 24 h until completely dry. These samples were then weighed, and the same process was repeated for the three feed types after being soaked in saltwater for 24 h. Three replicates were

performed. The obtained correction factors used for normalising the feed consumption data over dry matter content are presented in Table 3.

Table 3. Correction factors used to express the accessible feed and the collected feed remainder as dry matter.

Correction factor accessibility to feed as dry matter	Dry feed	0.774
	Dry feed + hydrolysate	0.750
	Lumpfish	0.159
Correction factor collected remainder of dry matter	Dry feed	0.363
	Dry feed + hydrolysate	0.376
	Lumpfish	0.130

2.3 Crab sampling and processing

The 24 RKCs that underwent the feed study and six reference RKCs were collected at the ARS, placed in dry storage in boxes with ice packs, and transported to Nofima (Tromsø), where they were stored overnight at 2 °C and processed the following day within 15 h of their arrival. The reference RKCs were kept in tanks together with other crabs of varied size and fed a different diet from the feed study group; they received wet feed consisting of several marine species excluding lumpfish. The carapace width (CPW) and length (CPL) of each RKC was measured with a calliper (precision ± 0.05 mm).

The procedure for processing the RKCs reflected the industrial processing and followed the steps described by Lian et al. (2021) with some modifications. Briefly, the processing started with registering the weight of the live RKCs, which were subsequently split into two clusters (i.e., three walking legs and the cheliped attached to a shoulder joint) using a butchering iron. Immediately after splitting, the hepatopancreas was collected from the carapace, weighed, placed in 50 mL test tubes, and stored at −40 °C until analysis. At the same time, each cluster was weighed and labelled using a T-bar tag (FD-94, Floy® tag & mfg) for traceability purposes. Afterward, the chelipeds were removed from the clusters and weighed. The clusters were cleaned from residues of entrails while being kept in a vertical position with the shoulder joint pointing downwards to facilitate drainage of the lymph fluid.

Afterward, the clusters underwent a "de-bleeding" step, which consists of immersing the clusters into a container with fresh water (100 L) at 4 °C for 2 h. Next, the clusters were drained for at least 30 min, and the weight of each de-bled and drained cluster was registered. Subsequently, the clusters were distributed into wire mesh baskets and cooked by immersion into a bath containing fresh water at 95 °C (±0.5 °C), continuously recirculated to increase the homogeneity of heat exchange and minimize cold spots. The cooking treatment lasted 12 min and aimed at reaching a core temperature of 92 °C in the merus of the second walking leg. Immediately after cooking, the clusters were cooled by immersion into a container with salted water added with ice (100 L, 3.5% w/v NaCl, sea salt, GC Rieber AS, Norway) for approximately 20 min until the core temperature was below 4 °C. The clusters were then drained for at least 30 min before their weights were registered.

After cooling and draining, one of the two clusters from each crab was selected, air-packed into plastic bags (thickness 80 µm, dimensions 220 × 600 mm, Finnvacum, Helsinki, Finland) closed with metallic clips, and stored in a climate chamber (BINDER GmbH, Tuttlingen, Germany) at 4 °C. Two days after processing, the clusters stored at 4 °C were reviewed for leg meat content analysis. The cooked leg meat was extracted from the clusters, placed in 50 mL test tubes, and stored at –40 °C until analysis.

2.4 Analytical determinations

2.4.1 Leg meat content

The meat content of the walking legs was measured in cooked clusters by digital analysis of images of a cross-section of the middle of the merus as described by Lian et al. (2021). The meat content was calculated as the relative proportion of the surface area of the leg cross-section occupied by meat. The leg meat content was determined in the merus of each of the three walking legs of the cooked clusters.

The images were analysed using ImageJ software, while the meat content was manually traced and sketched with a Wacom drawing board. A visual representation of a walking leg and the spots for merus cross-sectional cuts is given in Figure 4, whereas a visualization of the merus cross-sectional areas used for leg meat content estimation is shown in Figure 5.

Figure 4. Sketch of a walking leg (left) and merus (right).

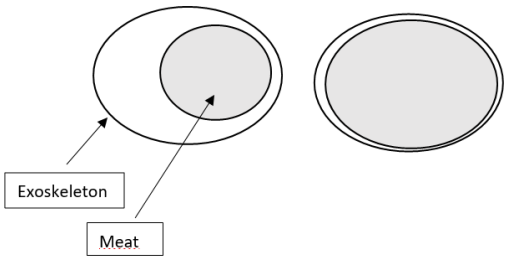

Figure 5. *Sketch of merus cross-sectional cuts in two different leg pieces. The left picture illustrates a leg with approximately 40% meat content, while the right picture shows a leg with approximately 95% meat content. The grey circle illustrates the area occupied by the meat.*

2.4.2 Thickness of the exoskeleton, hepatosomatic index, and cheliped index

The thickness of the exoskeleton was measured in the middle of the merus of the walking legs. It was measured using a caliper (precision ± 0.05 mm).

The hepatosomatic index (HSI) and the cheliped index (CI) were calculated as shown in Equation 2 and 3, respectively:

$$HSI = \left(\frac{W_{Hepatopancreas}}{W_{Crab}}\right) \times 100 \quad (2)$$

$$CI = \left(\frac{W_{Chelipeds}}{W_{Crab}}\right) \times 100 \quad (3)$$

where $W_{Hepatopancreas}$ is the weight of the raw hepatopancreas, $W_{Chelipeds}$ is the weight of the two raw chelipeds, and W_{Crab} is the weight of the corresponding whole raw RKC.

2.4.3 Cluster yield

The yields of the raw clusters (CY_{raw}) and of the cooked clusters (CY_{cooked}) were calculated as shown in Equation 4 and 5, respectively:

$$CY_{raw} = \left(\frac{W_{Raw\_clusters}}{W_{Crab}}\right) \times 100 \quad (4)$$

$$CY_{cooked} = \left(\frac{W_{Cooked\_clusters}}{W_{Crab}}\right) \times 100 \quad (5)$$

where $W_{Raw\_clusters}$ are the sum of the weight of the right and left cluster from the same crab after splitting, cleaning, de-bleeding, and draining, $W_{Cooked\_clusters}$ is the sum of the weight of the two cooked, cooled, and drained clusters obtained from the same crab, and W_{Crab} is the weight of the corresponding whole raw RKC.

2.4.4 Moisture and ash

To determine the moisture content in both cooked crab meat and raw hepatopancreas, the samples were dried in a thermostatic oven at a temperature of 105 °C for 48 h. The ash content was determined by placing the dried samples in a furnace at a temperature of 500 °C for 24 h. The moisture and the ash content were determined on samples generated from the crabs listed in Table 4, including samples obtained from moulted crabs.

2.4.5 Fatty acid analysis

Fatty acids (FA) analysis was performed by ALS Laboratories UK Ltd by Nuclear magnetic resonance (NMR) spectroscopy and Gas Chromatography-Flame ionization detector (GC-FID) with methods based on ISO 12966-2 and ISO 5509.

Three representative crabs from each dietary group were selected for the analysis (Table 4). The selected specimens had not undergone moulting at the time of sampling, and those chosen had the most linear feeding habits. Furthermore, both muscle and hepatopancreas tissues were tested in each crab to comprehensively evaluate FA composition. The tissues were mashed using an Ultra-Turrax® homogeniser (IKA® T-18 Basic Ultra Turrax, Staufen, Germany) before being sent to ALS Laboratories UK Ltd for analysis.

Table 4. Selected RKCs for moisture, ash, and fatty acid analysis for each diet group.

Diet group A	Diet group B	Diet group C	Diet group D	Reference group
3	6	2	5	x2
17	7	9	8	x3
19	18	10	23*	x4

*Note. *Fatty acid analysis only.*

2.5 Feed and energy intake

The daily feed intake (FI, g feed (kg crab)$^{-1}$ day^{-1}) was determined by calculating the difference between the amount of feed provided and the quantity of uneaten feed collected in each feeding effort. Since only two of the three feedings were sampled, the calculation of the weekly RKC's feed intake involved an estimation of the feed consumed on the third weekly feeding effort. This estimation was based on the assumption that, in a given week, each RKC consumed feed at the same daily rate calculated from the feed intake actually observed (i.e., sampled) in that week. The sum of the feed eaten in the two weekly feeding efforts was divided by four (days) multiplied by seven (days).

The energy value of each feed was expressed as kilojoule. The values of eaten feed (in gram of dry matter) were multiplied by the corresponding factor, 22.29 for dry feed and 9.06 for lumpfish (Table 2). The factor is the gross energy in one gram dry matter of each feed type. The energy intake was expressed as the amount feed eaten in the two feeding efforts that were sampled, per week.

2.6 Statistical analysis

The raw data and tables were processed in Microsoft Excel. The figures were generated by using the software GraphPad Prism (version 9.5.1 for Windows, GraphPad Software, San Diego, USA) and RStudio (2021.09.0: Integrated Development for R. RStudio, PBC, Boston). The values of the response variables measured on both the clusters generated from a single crab (i.e., CY_{raw} and CY_{cooked}) were averaged by crab. Differences between treatment groups were analysed by one–way analysis of variance (ANOVA) followed by a Tukey's HSD test and Tukey's test for multiple comparisons using the software R Studio. ANOVA was performed at a 95% confidence level ($\alpha = 0.95$) and considering each crab as an independent biological replicate.

3 Results

3.1 Observations during trial period

Morphological parameters and injuries of all the RKCs that participated in the feed study are given in Table 5. There was no mortality or loss of limbs in the trial period. It should be noted that six crabs, two in each the diet groups B, C and D completed a moult during the trial period. The double shell is measured on a scale from 0 to 2, with 0 indicating absence of a double shell, 1 corresponding to initiated formation of a double shell, and 2 representing the presence of a double shell.

Table 5. Morphological parameters, double shell grade, and injuries of the RKCs in the trial period.

Crab ID	Diet given	Initial crab weight (g)	Final crab weight (g)	Moulting date	Double shell	Initial injuries	Final injuries
3a	A	421	435		1	Missing L3	Missing L3
11a	A	597	625		2		
16a	A	378	395		1		
17a	A	572	576		0	Healed RC	Healed RC
19a	A	404	421		1		
22a	A	346	365		1		
Mean±SD: Initial crab weight 453±96 | Final crab weight 470±96 | CPL 8.2±0.1 | CPW 9.1±0.8							
4b	B	789	800		1		
6b	B	800	829		2		
7b	B	865	881		1		
12b	B	447	728	5 DEC	0	Healed R3	Healed R3
18b	B	349	362		1		
20b	B	469	685	14 NOV	0		
Mean±SD: Initial crab weight 620±203 | Final crab weight 714±170 | CPL 9.0±0.2 | CPW 10.1±0.1							
1c	C	379	598	07 NOV	0	Healed R2 L2	Healed R2 L2
2c	C	632	648		0	Healed L1	Healed L1
9c	C	349	366		1		
10c	C	753	768		0	Missing L3	Missing L3
13c	C	334	346		1		
21c	C	342	548	06 NOV	0		
Mean±SD: Initial crab weight 465±165 | Final crab weight 546±150 | CPL 8.3±0.9 | CPW 9.1±1.2							
5d	D	914	946		1		
8d	D	765	777		2		
14d	D	403	569	21 NOV	0		
15d	D	369	537	31 OCT	0	Missing R3	Missing R3
23d	D	346	354		0		
24d	D	340	348		2	Healed R2	Healed R2
Mean±SD: Initial crab weight 523±229 | Final crab weight 589±216 | CPL 8.6±1.1 | CPW 9.5±1.2							
x1	REF	NA	971		1	NA	
x2	REF	NA	446		1	NA	Healed R2
x3	REF	NA	512		0	NA	Healed RC
x4	REF	NA	469		0	NA	Healed R3 L2
x5	REF	NA	585		1	NA	Healed LC R1
x6	REF	NA	1052		1	NA	
Mean±SD: Crab weight 673±245 | CPL 9.2±1.0 | CPW 10.3±1.2							

Note. NA = not available. In the columns "initial injuries" and "final injuries", the letters L, R, and C indicate respectively left side, right side, and cheliped, whereas the numbers denote the limb article where the injury was observed.

Table 6 provides information about the changes seen in the crabs throughout the trial period. Some weight changes are seen in non-moulted crabs as well as in moulted crabs, but the large differences are only found in the moulted crabs. Only the moulted crabs have changes in CPL and CPW.

Table 6*. Variation (Δ) in the weight and carapace length (CPL) and width (CPW) of the RKCs during the trial period. Results are expressed as percentage variation relative to the values observed at the start of the trial period.*

Crab ID	*(Δ)* Weight (%)	*(Δ)* CPL (%)	*(Δ)* CPW (%)
3A	3.3	0.0	0.0
11A	4.7	0.0	0.0
16A	4.5	0.0	0.0
17A	0.7	0.0	0.0
19A	4.2	0.0	0.0
22A	5.5	0.0	0.0
4B	1.4	0.0	0.0
6B	3.6	0.0	0.0
7B	1.8	0.0	0.0
12B	62.9	22.9	19.1
18B	3.7	0.0	0.0
20B	46.1	13.3	15.1
1C	57.8	14.8	19.3
2C	2.5	0.0	0.0
9C	4.9	0.0	0.0
10C	2.0	0.0	0.0
13C	3.6	0.0	0.0
21C	60.2	15.8	18.8
5D	3.5	0.0	0.0
8D	1.6	0.0	0.0
14D	41.2	13.4	13.2
15D	45.5	16.7	17.4
23D	2.3	0.0	0.0
24D	2.4	0.0	0.0

In order to calculate the quantity of feed consumed by the RKCs, it was necessary to ensure excess feeding so that it was possible to sample the remainder unconsumed feed after 24 h from feeding. However, after a week of sampling, it was observed that several RKCs consumed their entire feed allotment. Therefore, since the quantity of feed they consumed could not be ascertained, the daily feeding regimes were adjusted on different occasions during the first weeks of the trial period to meet the individual appetite of the crabs (Table 7).

Table 7. Changes in the amount of feed given to RKCs expressed as g feed wet weight / kg crab / day.

Crab ID	Change to 7.5 g Dry feed 5 OCT	Change to 35 g Lumpfish 19 OCT	Change to 10 g Dry feed 19 OCT	Change to 20 g Dry feed 26 OCT
1c	*	*		
2c	*	*		
3a	*			
4b	*			
5d		*		
6b	*			
7b	*			
8d		*		
9c	*	*		
10c	*	*		
11a	*			
12b	*			
13c	*	*		
14d		*		
15d		*		
16a	*			
17a	*			
18b	*			
19a	*			
20b	*		*	*
21c	*	*		
22a	*			
23d		*		
24d		*		

A particular case occurred with RKC number 15d. One month after moulting, this RKC was observed to consume all the feed provided. Consequently, in the period 28th November–16th December 2022, additional feed (lumpfish) was provided to RKC 15d as reported in Table 8.

Table 8. Amount of additional feed (g feed wet weight / kg crab / feeding effort).and total feed (g feed wet weight / kg crab / feeding effort) provided to RKC number 15d in the period 28th November – 16th December 2022.

Date	Additional feed (g)	Total feed (g)
28 NOV	27.8	109.4
30 NOV	18.8	100.4
02 DEC	22.3	103.8
05 DEC	27.8	109.4
07 DEC	18.7	100.3
09 DEC	22.3	103.8
12 DEC	19.0	100.5
14 DEC	22.3	103.8
16 DEC	22.3	103.8

The water temperature at the ARS gradually decreased during the trial period from 9.6°C in the first week to 6.0°C in the 13th week (Figure 6). All RKC in the trial were subjected to the same water temperature.

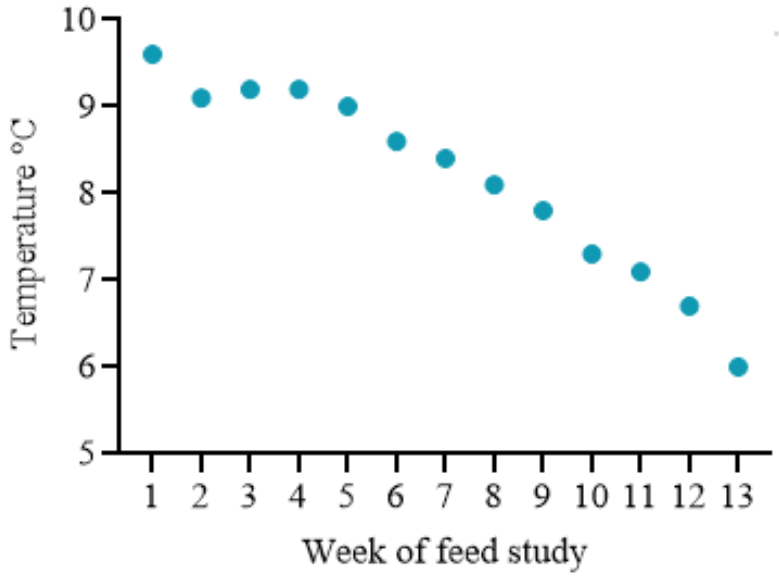

Figure 6. Water temperature at the ARS during the trial period.

3.2 Differences in feed intake between the diet groups

A visualization of the differences in the observed feed intake (expressed as dry matter) between the diet groups over the trial period of 12 weeks is given in Figure 7. The crabs in group B ate the highest amount of dry feed followed by the crabs in groups A and C. Group B started eating more (coated) dry feed than the other two groups at around week five and continued to eat more throughout the rest of the study. The crabs in group A slowly increased their feed intake until week five when their feed intake decreased. The crabs in group D ate more lumpfish than the crabs in group C throughout the 12-week period. Both groups had similar lumpfish intake until week two, after which group D's lumpfish intake was consistently higher than group C's. In group C, the consumption of dry feed decreased towards the end of the study as the consumption of lumpfish increased.

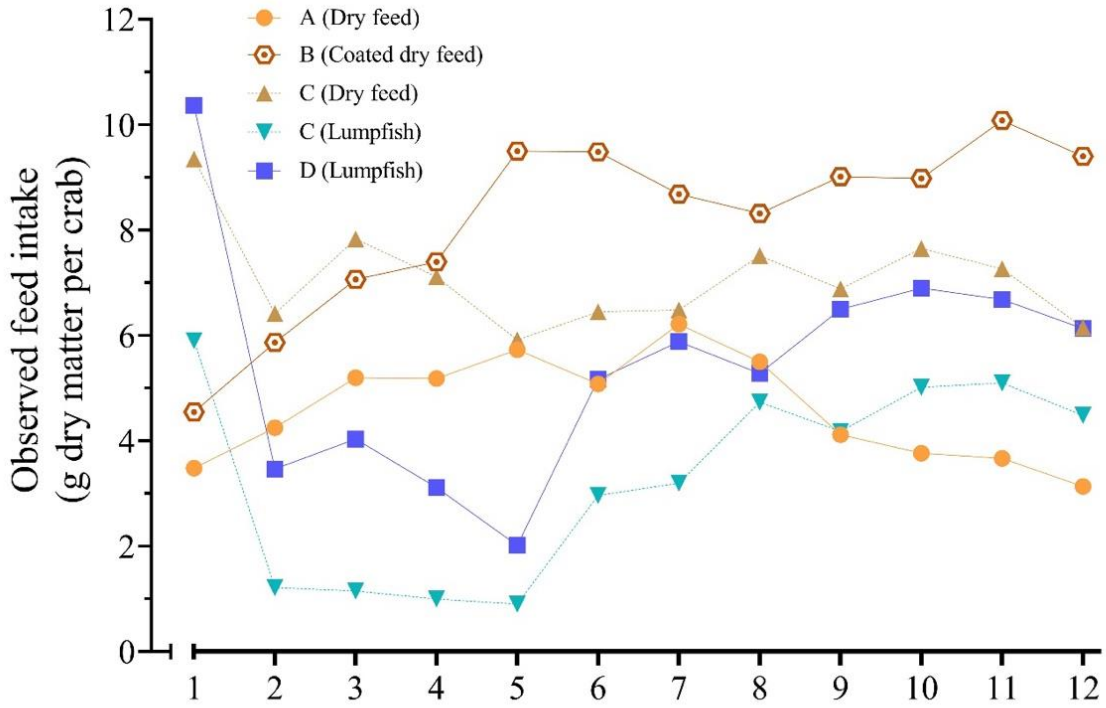

Figure 7. Observed feed intake over the 12-week trial period. Results are expressed as group mean in g of dry matter per RKC for a specific type of feed (dry feed or lumpfish).

The observed weekly feed intake (expressed as dry matter) for each crab specimen in each of the diet groups is illustrated in Figure 8. Although no moulting was observed in group A, notable differences in feed intake were observed, with crab 17a consuming the highest amount of feed and crab 22a showing the lowest feed intake. It is important to note that crab 22a was transferred to its designated chamber at the beginning of the trial, which may have influenced its feed intake. Furthermore, the feed intake of the crabs varied each week, indicating inconsistency in their feeding behaviour. In group B, crabs 12b and 20b were found to have moulted during the trial period, in week 7 and week 10, respectively. Notably, crab 20b exhibited the highest feed intake among all the crabs during the entire trial period. On the other hand, crab 4b was transferred to its designated chamber at the start of the trial, which may explain the delayed feed intake, which only commenced after week 5. In group C, crabs 1c and 21c moulted in week 6, which led to a reduction in their feed intake, as shown in Figure 8. Finally, in group D, the intake of lumpfish was initially high among all the crabs but dropped considerably and stayed low for a period of four weeks, before some of the crabs increased their feed intake whilst two continued to feed at relatively low rate during the whole trial. Notably,

crab number 14d and number 15d experienced moulting respectively in week 8 and 5, which resulted in an increase in their feed intake towards the end of the trial.

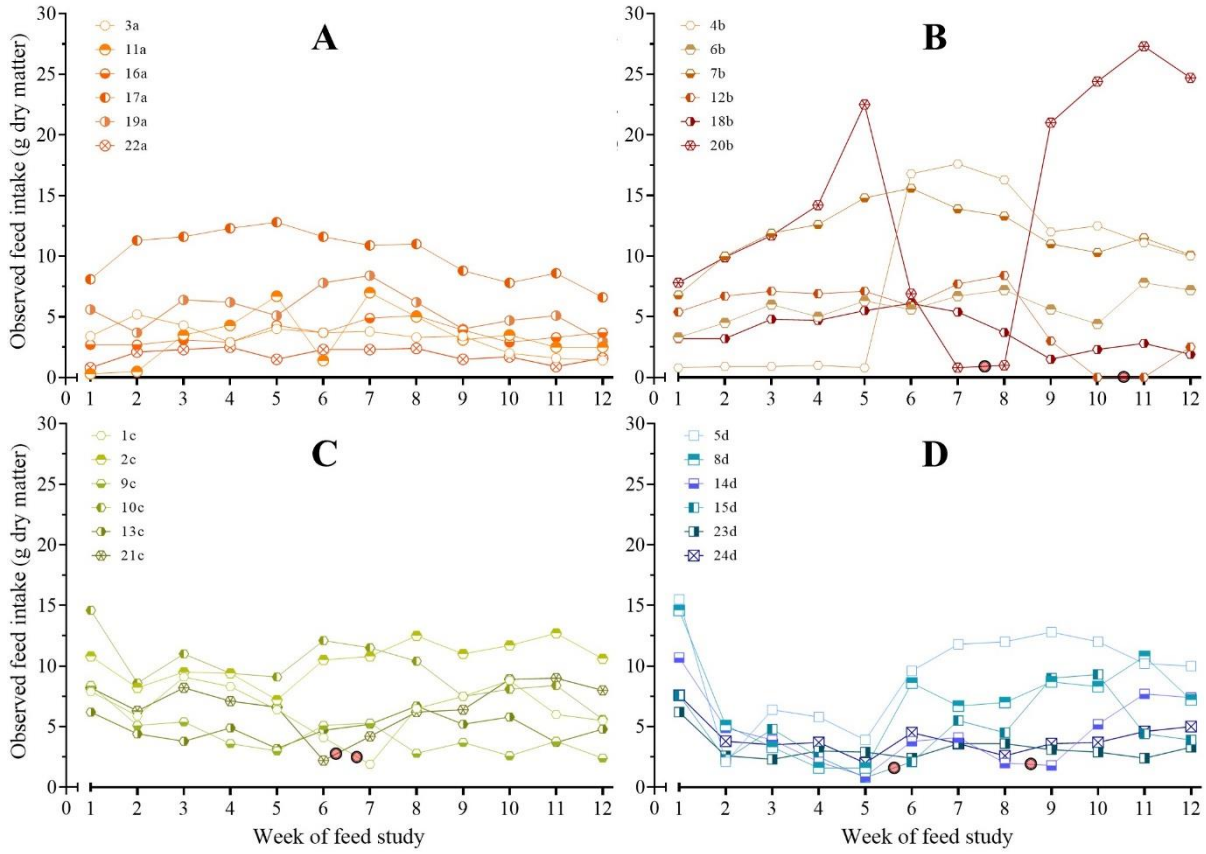

Figure 8. Observed weekly feed intake during the 12-week trial period for each RKC specimen in the diet groups (indicated by superscript letter). Results are expressed as g of dry matter per RKC for the sum of the two types of feed (dry feed and lumpfish). Red circles with black borders indicate time of moult completion for corresponding RKCs.

Figure 9 show the average feed intake observed weekly per crab. The results indicate that group B had the highest feed intake, followed by groups C, D, and A. The ANOVA test results show that there are no significant differences in average feed intake observed weekly between the diet groups.

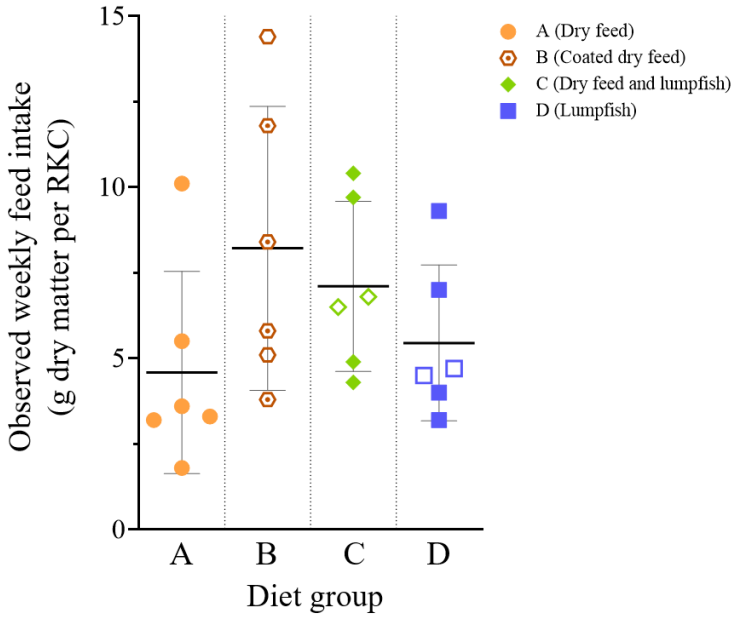

Figure 9. Observed average weekly feed intake over the entire 12-week trial period for each RKC specimen in the diet groups. Results are expressed as g of dry matter per RKC for the sum of the two types of feed (dry feed and lumpfish). Specimens that have completed a moult during the trial period are depicted with blank markers. Diet group means (± SD) (calculated including moulted specimens) are indicated by horizontal lines and error bars.

Figure 10 illustrates the average feed intake observed weekly per crab only considering the specimens that did not complete a moult in the trial period. The feed intake varied between the different diet groups, with group B having higher mean feed intake, than group C, D and lastly group A. An ANOVA revealed that no statistically significant difference between the mean feed intake of the four diet groups were found.

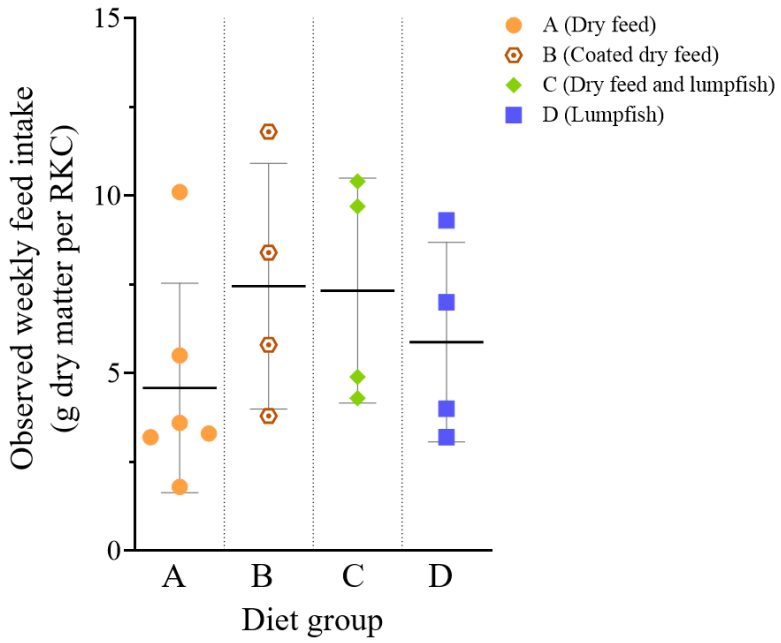

Figure 10. Observed average weekly feed intake over the entire 12-week trial period for RKC specimens that did not complete a moult in the trial period. Results are expressed as g of dry matter per RKC for the sum of the two types of feed (dry feed and lumpfish). Diet group means (± SD) (calculated excluding moulted specimens) are indicated by horizontal lines and error bars.

3.2.1 Weight start compared with amount eaten

In Figure 11 there is a positive relationship between the starting weight of the crab and the amount of feed they consume. This suggests that larger crabs tend to consume more feed than smaller crabs. However, there is some variability in the data, indicating that the relationship is not perfect. In Figure 11 a line of best fit is included, which is a linear regression model that estimates the average relationship between the starting weight and mean feed eaten, holding constant the type of crab. The slope of the line indicates the average increase in mean feed eaten associated with a one–gram increase in starting weight. The intercept of the line indicates the average amount of feed consumed by a crab of weight zero (which is not biologically meaningful in this context). A model summary provides information on the linear regression model that was fit to the data. The intercept is not significant (p–value = 0.35849), but the slope is significant (p–value = 0.00254 **), suggesting that there is a significant positive relationship between the start weight and mean feed eaten of the crabs. The "F–statistic" and "p–value" provide information on the overall significance of the regression model. In this case, the F–statistic is 11.6 with a p–value of 0.002539, indicating that the model is significant as a whole.

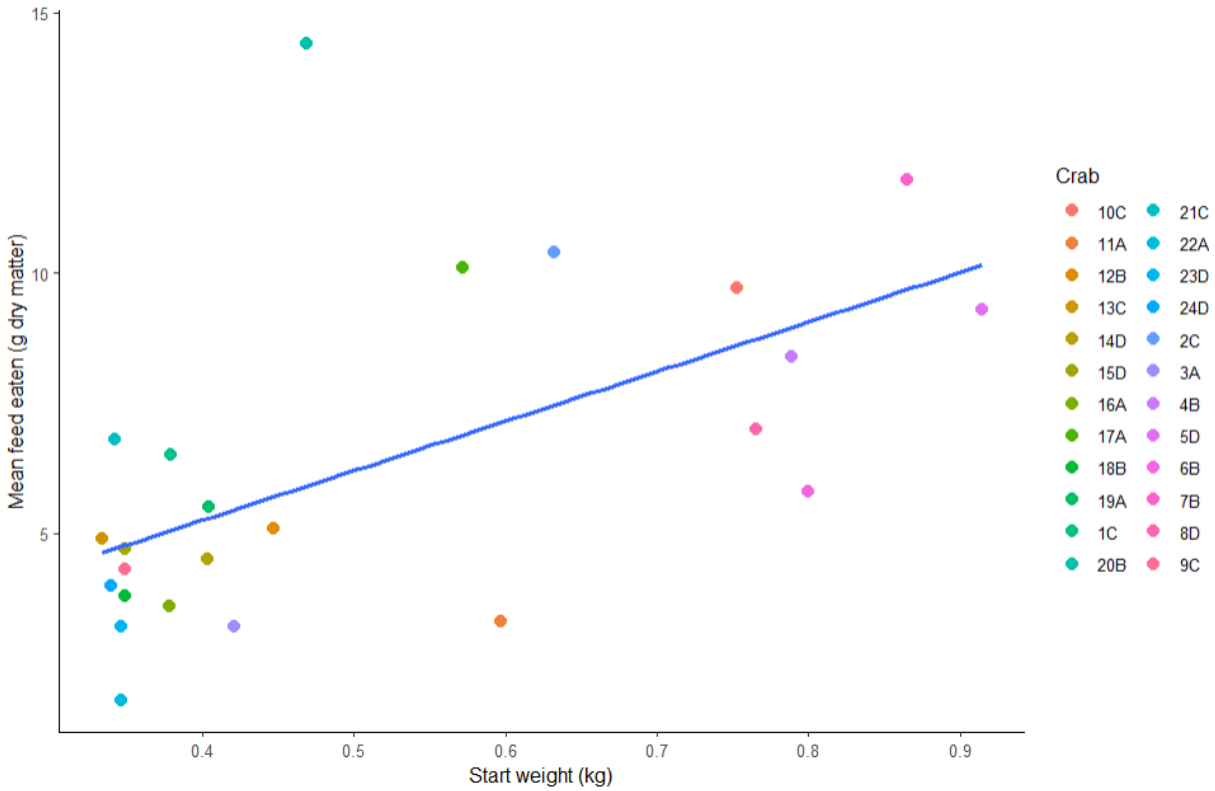

Figure 11. The scatter plot shows the relationship between the starting weight of each crab and the amount of feed (in dry matter) they consumed on average over 12 weeks of feeding. Each data point represents one crab.

3.3 Feed intake and energy intake

Table 9 presents the estimated FI, which quantifies the amount of feed consumed per feeding by each RKC in the study. It is the expected feeding effort per day per week. The table also reports the standard deviation of the FI. It should be noted that the feeding behaviour of the RKCs varied throughout the trial period. However, the table presents an estimated average of the mean amount of feed consumed per crab over the course of the 12-week feed study. Furthermore, the standard deviation of the FI for moulted RKCs tends to be greater than that of non–moulted RKCs. An ANOVA was conducted and showed no statistical differences between the FI of the diet groups.

Table 9. Weekly average FI (g feed dry matter kg^{-1} day^{-1}, ± standard deviation) over the entire 12-week trial period for each RKC specimen in the diet groups. The mean for each group is also presented with standard deviation.

Crab ID	Mean FI Diet A	Crab ID	Mean FI Diet B	Crab ID	Mean FI Diet C	Crab ID	Mean FI Diet D
3a	1.8±0.6	4b	2.7±2.1	1c	4.2±1.3	5d	2.6±1.0
11a	1.4±0.9	6b	1.8±0.4	2c	4.0±0.6	8d	2.3±1.2
16a	2.4±0.5	7b	3.4±0.7	9c	3.1±1.2	14d	2.8±1.7
17a	4.2±0.8	12b	2.7±1.5	10c	3.0±0.7	15d	3.1±1.7
19a	3.4±0.9	18b	2.7±1.0	13c	3.6±0.7	23d	2.3±0.7
22a	1.2±0.4	20b	7.7±4.8	21c	5.0±1.4	24d	3.0±1.0
Mean	2.4±1.1	Mean	3.5±1.9	Mean	3.8±0.3	Mean	2.7±0.4

Calculated gross energy equivalent to what the crabs have eaten in the two feeding efforts that were recorded, expressed per week is presented in Table 10. The values of eaten feed (in gram of dry matter) were multiplied by the corresponding factor: 22.29 for dry feed and 9.06 for lumpfish. The factor is the energy in one gram of dry matter. The mean energy intake in kJ by each crab per week with standard deviation is presented in Table 10. An ANOVA was conducted and showed a statistical difference between the energy intake between the diet groups (p-value=9.32e-14 ***). The Post-hoc tests using Tukey's honestly significant difference (HSD) test indicated that there are significant differences in p-value between diet groups A and B (p–value =0.0018970), D and A (p–value =0.0000363), C and B (p–value=0.0438556), D and B (p–value =0.0000000) and D and C (p–value =0.0000003). These results suggest differences in energy intake between the groups.

Table 10. Observed weekly average energy intake (kJ, ± standard deviation)) over the entire 12-week trial period for each RKC specimen in the diet groups. The mean for each group is also presented with standard deviation.

Crab ID	Mean energy intake	Crab ID	Mean energy intake	Crab ID	Mean energy intake	Crab ID	Mean energy intake
3a	164.0±54.0	4b	237.3±189.2	1c	277,1±119.3	5d	92.6±37.9
11a	125.1±76.6	6b	162.1±35.7	2c	208.7±33.7	8d	82.3±42.5
16a	211.7±46.3	7b	304.4±60.1	9c	176.5±83.4	14d	102.3±62.0
17a	378.2±72.3	12b	243.8±135.9	10c	182.7±60.6	15d	111.4±61.7
19a	304.4±84.5	18b	240.6±92.2	13c	243.8±51.5	23d	83.7±26.4
22a	110.8±34.1	20b	682.3±427.9	21c	298.3±115.7	24d	107.2±35.3
Mean	215.7±96.8	Mean	311.7±170.8	Mean	243.2±47.8	Mean	96.6±11.2

3.4 Quality parameters

3.4.1 Meat content and processing yield

Figure 12 can be used to compare the central tendency and spread of meat content across the different diet groups, as well as to identify any potential outliers. When conducting a one–way ANOVA test no significant differences in meat content between either of the diet groups or the reference group were found, suggesting that the different diets did not have a significant effect on the meat content of the crabs.

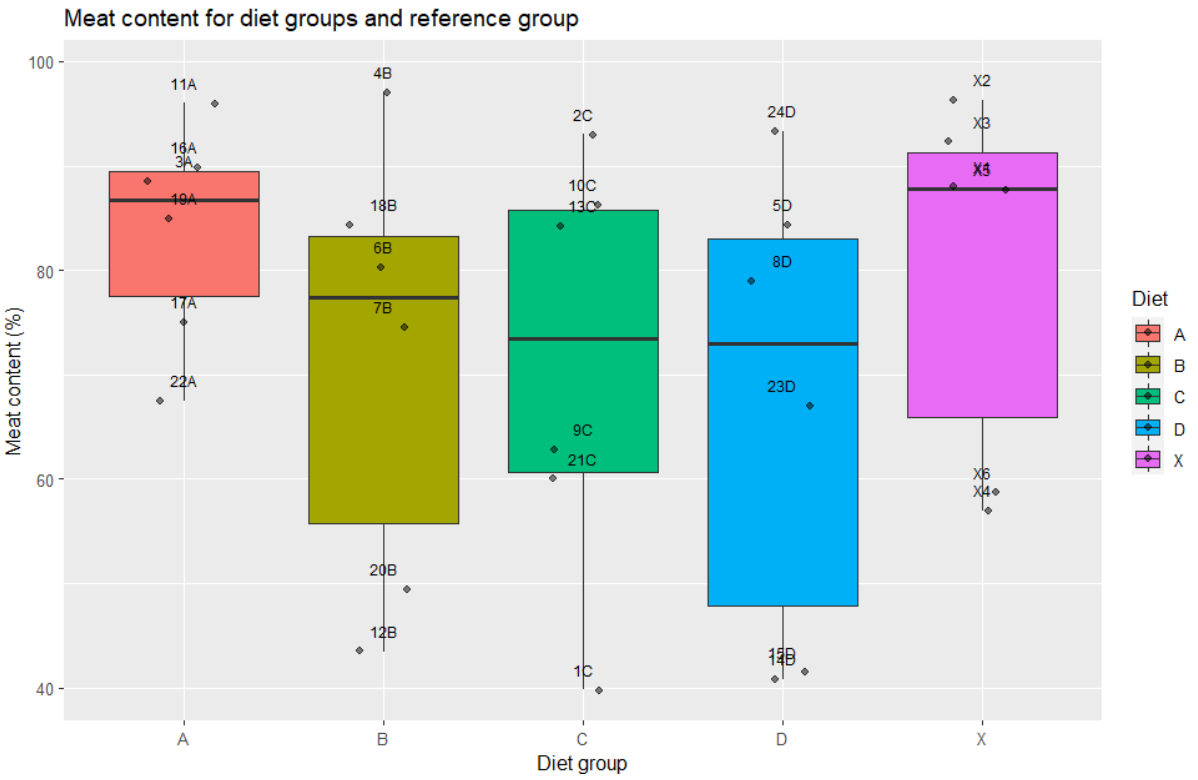

Figure 12. The box plot shows the distribution of meat content for each diet group, with individual observations plotted as jitter points on top. The box in the middle of each group represents the interquartile range (IQR) of the data, with the line inside the box representing the median. The whiskers extend to the minimum and maximum values within 1.5 times the IQR from the box, and any points beyond the whiskers are considered outliers. The fill colour of each box corresponds to the diet group. X represent the reference group.

The data has been subset to exclude the reference group and crabs that have moulted, and Figure 13 with individual observations visually explore the relationship between diet and meat content. Figure 13 allows for comparison of the distribution of meat content between different diet groups, without the moulted crabs and the reference group, and to identify any potential outliers. A one–way ANOVA test was performed to determine if there were significant differences in meat content between the diet groups. The results show that there is no significant difference between the diet groups, suggesting that the different diets did not have a significant effect on the meat content of the crabs.

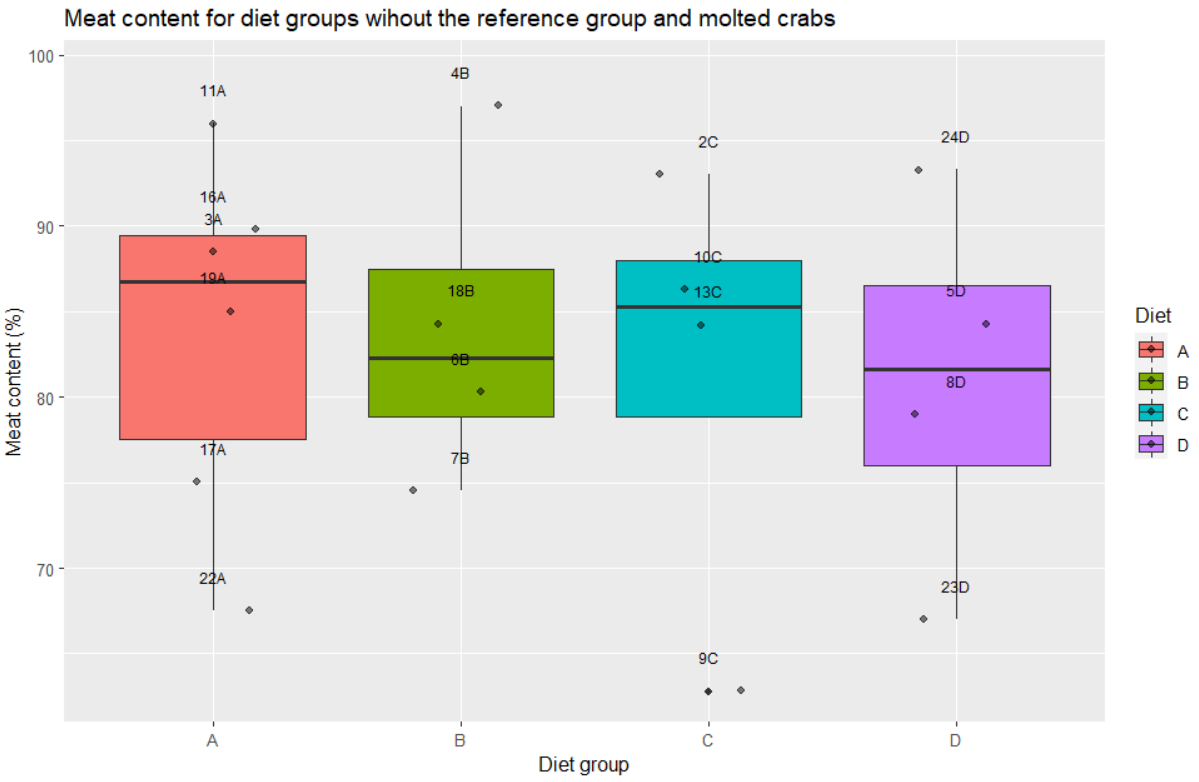

Figure 13. The box plot shows the distribution of meat content (%) across different diet groups, after excluding the moulting crabs and the reference group. Each box represents the interquartile range (IQR) of the data, with the median value shown as a horizontal line inside the box. The whiskers extend to the most extreme data points that are within 1.5 times the IQR from the box. The individual data points are also plotted on the graph as jittered points, with the transparency (alpha) set to 0.5 to help visualize the density of the data.

3.4.2 Processing data

Table 11 present the data calculated from the processing data. Although the fat content in the hepatopancreas was analysed, the values obtained were not accurate owing to the limited sample size. An ANOVA was conducted showing significant differences in cooked CY (p–value =6.91e-13 ***) and in exoskeleton thickness (p–value = 6.19e-07 ***). It was followed with a Tukey's test for multiple comparisons which found significant differences in the CY_{cooked} between the moulted crabs and all the groups with a p-value of $p < 0.001$ for all groups. Significant differences were also found between the moulted crabs and all other groups in the exoskeleton thickness. The p-value was $p < 0.001$ for all groups. Significant differences were not found for any of the other parameters in the table.

Table 11. Results are expressed as mean values (± standard deviation) in %, except for the thickness of the exoskeleton which is presented in mm.

(%)		Diet group A	Diet group B	Diet group C	Diet group D	Reference group	Moulted crabs
HSI		5.2±1.0	4.7±0.4	5.8±0.5	5.2±0.8	5.3±0.8	4.5±0.6
CI		3.7±0.3	3.6±0.2	4.4±1.5	3.7±0.4	3.6±0.3	4.2±0.7
Cooked leg meat:							
	Moisture	80.0±0.8	79.8±0.6	79.1±0.6	79.6±0.4	78.3±0.2	78.3±5.4
	Fat	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0	NA
	Ash	1.3±0.3	1.4±0.4	1.4±0.2	1.9±0.6	1.5±0.5	1.8±0.5
Raw hepatopancreas:							
	Moisture	66.3±5.9	66.6±0.8	60.2±5.1	57.3±3.4	61.9±2.4	68.8±0.7
	Ash	1.5±0.3	1.3±0.4	1.6±0.3	0.9±0.2	1.7±0.1	1.6±0.1
CY_{raw}		27.3±1.2	27.7±0.5	26.6±1.8	27.4±0.6	26.7±0.6	26.3±0.4
CY_{cooked}		27.3±1.1	26.9±1.1	27.9±0.7	26.3±1.3	26.9±0.8	17.7±1.3
Thickness exoskeleton		0.4±0.0	0.5±0.0	0.4±0.0	0.4±0.1	0.4±0.1	0.2±0.0

Table 12 illustrates the Fatty analysis (FA) conducted with the selected crabs (Table 4). The most important components of the analysis saturated fatty acid (SFA), monosaturated fatty acid (MUFA), poly unsaturated fatty acid (PUFA) and sum omega 3 fatty acid (∑n–3) is presented in the table. It is presented as mean per group for both tissues, cooked crab meat and raw hepatopancreas. Among the FA assayed in the leg meat of the RKCs, PUFAs were present in the highest proportion, followed by MUFAs and SFAs. The FA profile of the hepatopancreas of the RKCs was characterized by MUFAs found in highest proportions, followed by PUFAs and lastly SFAs. In the hepatopancreas in diet group C and D the ∑MUFA is observed to be lower than in other groups but the ∑PUFA is observed to be higher. ANOVA tests were conducted and followed by a Tukey's test to compare the statistical differences between the different diet groups and the reference group in terms of FA types and omega-3 content. The test was performed for both the cooked crab leg meat and the raw hepatopancreas. No significant differences were found in the cooked leg met. However, in the hepatopancreas differences was found in ∑MUFA (p= 0.000665 ***) and ∑PUFA (p= 0.000269 ***). In ∑MUFA there was statistical differences between group C and A (p=0.0476005), D and A (p=0.0007289), D and B (P=0.0018103) and reference and D (p=0053567). In ∑PUFA significant differences was found between reference group and A (p=0.0037896), reference and B (p=0.0025260), reference and C (p=0.0008092) and reference and D (p=0.0002053).

Table 12. Fatty acid composition (% of the total detected fatty acids) of cooked leg meat and raw hepatopancreas. ∑n–3 (sample) is presented as mg/100g per sample. Results are expressed as mean values (± standard deviation).

%	Diet group A	Diet group B	Diet group C	Diet group D	Reference group
Cooked crab meat					
∑SFA	19.0±0.4	19.4±0.5	19.3±0.6	20.2±0.7	18.8±0.3
∑MUFA	33.6±0.8	33.1±1.5	32.8±1.8	31.6±0.6	35.3±1.3
∑PUFA	47.4±0.4	47.5±1.4	47.9±1.3	48.1±0.1	45.9±1.0
∑n–3 (sample)	49.3±18.9	48.7±17.3	48.7±.15.8	60.0±17.0	74.0±1.6
Raw hepatopancreas					
∑SFA	15.9±1.0	15.8±0.6	16.3±1.4	16.3±1.4	14.4±1.0
∑MUFA	58.4±0.7	57.7±0.7	51.9±1.1	51.9±1.1	56.9±1.4
∑PUFA	25.8±1.0	26.5±0.1	31.7±1.1	31.7±1.1	28.7±0.5
∑n–3 (sample)	5232.3±4665.5	3688.7±756.1	6671.0±1187.2	5692.0±2908.7	10336.3±4531.8

4 Discussion

The primary objective of this study was to address an ecological problem while achieving economic benefits. The RKC is a valuable resource but poses a significant threat to the ecosystem because it is an invasive species. To mitigate this issue, this study proposes harvesting juvenile RKC and rearing them to commercial size by feeding them with lumpfish, an otherwise poorly utilized resource. This approach ensures the preservation and cultivation of the RKC while alleviating the stress on the ecosystem. Typically, the aquaculture industry incurs expenses in disposing of lumpfish after they reach sexual maturity (Nøstvold et al., 2016). By utilizing the lumpfish as a feed resource, the industry hopefully can avoid these expenses. The proposed solution offers an opportunity to address ecological and economic problems sustainably while promoting resource enhancement.

4.1 Feeding behaviour and ecosystem effects

The results indicate that the RKC feeding on dry feed coated with lumpfish (diet B) had a consistently increased intake throughout the trial period (Figure 8). This indicates the coated feed's potential to stimulate the RKC's appetite. Interestingly, the group receiving only lumpfish (diet D) showed a high feed intake in the first week, which decreased abruptly. The group then had a slight increase after week 5, but still with low feed intake. A similar trend was observed for the group receiving only dry feed (diet A). This might suggest that the RKC prefers a varied diet rather than a single feed type. For the group receiving both lumpfish and dry feed (diet C), the dry feed intake decreased over time, while that of lumpfish increased. There are few, if any, systematic studies on the nutritional requirements of the RKC. The dry feed used in this experiment is based on general studies of the nutritional needs of other crab and lobster species (D'Abramo et al., 1997). Therefore, there may be species-specific differences in the animals' nutritional requirements that Nofima's diet has not captured. Figures 9 and 10 demonstrate that

diet B was the most attractive, followed by diet C. Diet B was added a lumpfish hydrolysate consisting mainly of low-molecular-weight peptides and free amino acids. These are known from literature to function as attractants, which in this case, can trigger a better appetite in crabs (Stevens, 2014). In their natural habitat, RKC feed on various species, and a diet consisting of a single food source may not be sufficient to meet their dietary needs, which could explain why they seemed to prefer the mixed diets.

The FI of the RKCs is presented in Table 9. The table suggests that diet group C had the highest FI, followed by diet group B, and then diet groups D and A. However, no statistical difference between the diet groups was found. Table 10 reveals significant differences in energy intake. The RKCs fed with diet B had the highest intake of energy, followed by diet C and diet A. On the other hand, the RKCs receiving diet D tended to consume less energy than the other diet groups. Due to the unclear nutritional requirements of the RKCs, it is difficult to draw a definitive conclusion regarding the efficiency of any of the diets. Nevertheless, it appears that diet D alone may not be adequate for satisfying the nutritional needs of the RKC. The energy intake of diet group D is lower than what was expected and may be too low for the RKC over time. Several studies have been conducted on FI, also referred to as feed ration (FR), in RKC, with varying results. Zhou et al. (1998) studied ovigerous females, juvenile females, and mature males for four months and calculated the mean FI using the wet weight of squid (*Loligo opalescent*). The mean FI values were 51.3±12.9 g^{-1} kg^{-1} day^{-1} for ovigerous females, 54.0 ± 16.8 g^{-1} kg^{-1} day^{-1} for juvenile females, and 46.9±13.7 g^{-1} kg^{-1} day^{-1} for adult males. These values are higher than those observed in this trial, where the mean FI for groups A–D were 2.4±1.1, 3.5±1.9, 3.8±0.3, and 2.7±0.4, respectively (Table 9). However, it is important to note that Zhou et al. (1998) expressed the FI in wet weight, not dry matter, and used crabs of different sizes and sexes. Stevens (2012) fed juvenile crabs, with a mean size of 25–30 cm, with squid and found significant differences in FI before, during, and after moulting, which is consistent with the results of this study (Figure 8). Although the FI in Stevens (2012) study was lower than in this trial, direct comparisons cannot be made due to differences in temperature, feed, and crab sizes. Siikavuopio and James (2015) fed adult RKC with a diet of herring (*Clupea harengus*) for 110 days at different temperature regimes. The FI registered was closer to the FI seen in this trial than in other studies. It is also important to consider that feeding rates vary with crab size and temperature; therefore, comparisons between different studies should be made cautiously. The purpose of the FI estimate was to provide an illustration of the feeding behaviour of the crabs; the estimate is based on two feedings per week and not three. The FI is, therefore, not an absolute value but an estimate of what can be expected of the juvenile RKC to consume in dry matter per kg of weight. Overall, the varying results from different studies highlight the need for further research on nutrition and FI in RKC, which is crucial for their cultivation and ensuring adequate nutrition while keeping reasonable production costs.

The estimated daily energy intake of the RKCs during the feed study is presented in Table 10. The results suggest that the RKCs in diet group B had the highest intake of energy, closely followed by diet group C, A and lastly, group D. Lee and Lawrence (1997) observed that crustaceans tend to have a small proventriculus volume that fills up quickly but is able to clear 75% of a meal within an hour. This suggests that the RKC may require frequent, smaller meals

rather than large portions at one time. Additionally, Stevens (2012) noted that feed intake may be regulated by stomach fullness rather than caloric intake. This may explain why the crabs in the trial were unable to eat all the lumpfish provided, even though the amount given was estimated based on their caloric needs. The volume of lumpfish may have inhibited the crab's ability to eat more, whereas the smaller volumes of dry feed may not have filled their stomachs to the same extent. Stevens (2012) found that the crabs in their experiment, held under similar conditions as this study, ate approximately 1.51 g of wet biomass, converting to 2.41 kcal per feeding with squid. However, the energy intake of the crabs in the current trial, as presented in Table 10, indicates a higher daily estimated caloric intake compared to the observations made by Stevens (2012). However, it is important to note that the different experimental setups, varying crab sizes and sexes, and different conditions and diets make it challenging to draw conclusions about feeding behaviour, energy intake and FI.

Diet D has an estimated energy intake of 96.6±11.2 kJ, which may imply how much the juvenile RKC can eat of benthos. A study by Pavlova (2008) highlighted the top-down effect of juvenile RKC on soft-bottom fauna, which may have a negative impact on local biomass. The benthic-pelagic coupling is vital to the overall functioning of the ocean due to the exchange of energy and nutrients throughout the water column. Burrowing species play a crucial role in facilitating this process. While the RKC forages in the sediment and moves it around, it does not burrow into the sediment to facilitate oxygen and nutrient movement. By removing the juvenile RKC from the system, these animals may be spared. The impact of the RKC on benthic-pelagic coupling remains inadequately studied, and further research is necessary to understand the potential changes to this process and their implications.

The amount of each feed type eaten is presented in Figure 7. The crabs who moulted while receiving lumpfish, increased their lumpfish intake after moulting. Ageeva et al. (2021) described the mineral composition in farmed lumpfish and found the Ca content to be 232 mg^{-1} $100g^{-1}$. The dry feed used in this feed study had no added Ca, but there is an unknown quantity in the different components of the feed. The increasing feed intake of lumpfish, seen in Figure 7, may be due to the filling of Ca storage, or it can be the preferred taste of the fish that explains the increasing feed intake. Greenavvay (1985) researched the calcium balance in crustaceans. The requirements for calcium in crustacean species are widely different, even between near-related species. The requirement for calcium in RKCs has not been studied, and due to the differences in nutritional requirements Britayev et al. (2010) saw that calcium-rich species played a key role in the diet of small RKCs who moulted frequently. Large marine crustaceans with large exoskeletons require a large amount of calcium. When RCKs moult calcium is lost to the environment, the crabs mainly replace the mineral through the water, but also through foraging calcium-rich species. The amount of foraging needed to fill up the storages are unknown. More research on the mineral requirements of the RKC must be done to better assess what feed should be given after moulting.

The underlying reasons for the observed variations in feed intake among RKC remain elusive, with a lack of empirical evidence to explain this behaviour. Despite extensive research on RKC feeding patterns, knowledge of the factors affecting feed intake in RKC is still limited.

However, by investigating RKC group dynamics in natural environments, valuable insights into individual feeding behaviours may help to understand observations in laboratory settings. Dew (1990) described the podding behaviour of the RKC in nature. The pods consist of individuals of approximately the same size and age class. Competition between crabs of different sizes may, therefore, not be apparent. The reference crabs used in this trial was kept in champers with crabs of different size and age class. Differences in feed intake between these crabs may have occurred if the larger crabs were entitled to the feed first. This theory may explain some of the variations seen in meat content in Figure 12. Other explanations for the variations may be that crab number x4 and x6 had moulted, but data confirming that are unavailable. Stoner, Ottmar and Haines (2010) observed cannibalism between 1– and 0–year–old RKC. They observed that the 1–year–old RKC was an effective predator of the smaller crabs, especially during moulting. Siikavuopio et al. (2016) found that the risk of cannibalism increased with increasing stocking densities. In situations where a high population density of crabs exists within a confined space, such as in chambers, the incidence of cannibalism, mortality, and injuries may be expected to increase. However, in the present feed study, the crabs were housed in individual chambers, and no such adverse effects were observed, but it should be considered when upscaling such an experiment.

Figure 11 illustrates the relationship seen between weight and feed eaten. Although individual differences are seen, the trend suggests that larger crabs ate more. If comparing Table 9 with Table 5, the size of the crab can be compared with the FI. Some of the smaller crabs ate more than the large crabs, but it was not a clear trend. Jewett and Feder (1982) found that juvenile RKC of size smaller than 140 mm exhibits a greater feeding rate compared to adults. This can be attributed to their high appetite and feeding behaviour, which involves grasping and tearing prey resulting in excessive spillage. Given this, removing juvenile RKC from oceanic ecosystems could have benefits. Since all crabs in this trial were considered juveniles, it was not possible to compare feeding behaviour between juveniles and adult RKCs.

The RKC's high temperature tolerance suggest that it may expand further south down the Norwegian coast. Figure 6 shows that the temperatures decreased steadily with almost 4 °C over the trial period. However, Falk-Petersen et al. (2011) and Christiansen et al. (2015) described the preference the RKC had for temperatures between 2–7 °C. Shirley and Korn (1989) suggested that moulting happened earlier if the temperature was higher. The temperature during the first part of the feed study was above the optimal temperature for the RKC, which may be one of the reasons for the unexpected moulting observed. Under ideal conditions, a lower temperature regime and/or constant temperature could have been used, allowing a more robust interpretation of the data. However, cooling of water is expensive and would have increased the production costs and, therefore, not be an option for commercial production. RKC feed intake may have been affected by a cooling trend in line with previous findings by Stoner, Ottmar and Haines (2010). In laboratory studies, they observed that rising temperature increased metabolism and feed intake, when keeping 0–1–year–old RKC. Stevens (2012), Lian et al. (2022), and Siikavuopio and James (2015) also reported an increase in the RKC's metabolism in higher temperatures, resulting in increased feeding. With the estimated

distribution of the RKC extending to Trondheim[2], the potential impact of the species on ecosystems in a warming climate may be substantial. The crab's generalist feeding behaviour and its opportunistic approach ensure the crabs ability to adapt to changing conditions and exploit available resources.

In this trial, it was observed that the RKC resumed eating at various times after moulting as well as at different rates and amounts (Figure 8). The differences in feed intake during moulting seen in this feed study are comparable with studies on other crustaceans. Lipcius and Herrnkind (1982) observed that the spiny lobster *(Panulirus argus),* where declining feed intake before moulting and ceased intake during moulting. After moulting, the lobster was observed to eat more than before moulting. This correlates with the trends of the moulted crabs in this trial (see Figure 8). Takeuchi (1968) investigated the feeding habits of RKC off the west coast of Kamchatka. It was noted that the feeding habits were altered around the moulting time of the RKC. The feed composition and the FI differed from the rest of the year. This change was noted either due to the migration patterns, different prey at different depths, or the preference of different feed sources during the moulting period. Individual differences in feed intake between RKC in laboratory experiments have also been observed by Zhou et al. (1998), who reported changes in FI before, during, and after moulting. The individual differences observed between crabs of the same size, gender, and diet may make it difficult to know exactly how to manage the RKC optimally. Figure 8 also visualizes the different feeding patterns of the crabs. For example, RKC number 20b ate significantly larger amounts of feed than any other crab. RKC number 4b did not eat until week five, but then ate large amounts of feed. It is important to be aware of individual differences in feeding behaviour to ensure adequate care of the animals. During the trial, it was necessary to regulate the amount of feed given to the RKCs due to their unexpectedly high feeding activity. After one week of sampling, it was apparent that the RKCs eating dry feed ate all their allotment of feed. To ensure the RKC had enough feed, the amount was regulated during the feed study (see Tables 7 and 8).

4.2 Sampling and correction factor

The RKC in the study received feedings three times per week. However, the analysis was only conducted on two of these feedings. Figures 7-11 are based on the data obtained from these two feedings, resulting in an underestimation of one feeding. Assuming that two out of three meals per week gives a representative image of the total feed intake, these figures offer valuable insights into the feeding patterns of the RKC, revealing differences in feeding behaviour among individual crabs and across diet groups. When the feed was collected 24 hours after feeding, the collected sample contained faecal matter, which may have resulted in a slightly underestimated calculated FI for the RKC.

In this study, a correction factor was employed to account for the high moisture content of the feed, as indicated in Table 3. However, the lumpfish supplied to the RKC contained a significant

[2] Citation Carsten Hvingel, Head of Research at Norwegian Institute of Marine Research (IMR) (*Havforskningsinstituttet*), newsletter November 2019.

amount of water that was not considered part of their feed. As a result, the use of the correction factor resulted in certain RKC being assigned negative values for the amount of feed consumed, which suggested that they had not consumed any feed. These negative values were recognized as being inaccurate and were subsequently adjusted to zero. While the RKC may have consumed more feed than estimated, the use of correction factors was deemed necessary due to the nature of the feed. Without these correction factors, a considerable number of crabs would have been assigned negative values due to the moisture absorption of the feed during the 24-hour exposure to seawater.

4.3 Quality parameters, yield, and morphological data

Although the main focus in this thesis is placed on sustainability and resource biology, some quality and morphological parameters are included to ensure that the diets lead to a high-quality product. The studied parameters included are crab morphological features, such as the thickness of the exoskeleton, the hepatosomatic and cheliped index, and the contents of hepatopancreas, leg meat, and FA.

Our results showed no significant differences in meat content between the diet groups. However, Figure 12 indicates notable variations in meat content among the groups containing moulting crabs. Specifically, the crabs with the lowest meat content in diet groups B, C, and D had undergone moulting. While it is possible that reference crabs x4 and x6 may have also moulted, data are not available to confirm this. Figure 13 presents the mean meat content for each diet group, excluding moulting crabs and reference crabs. No significant differences between the groups were found, consistent with the results of James et al. (2013), who fed RKC for nine weeks with different diets. This trend may suggest that the trial period was too short or that the feed provided, while diverse, may have been nutritionally adequate for RKC. Table 5 presents data on RKC with double shells, which may indicate which crabs were near moulting. Some crabs with double shells had the highest meat content in their respective groups. These finding suggests that these crabs were likely ready for moulting, as their exoskeletons were full, and they required more space to accommodate growth. In his study, Dew (1990) observed the moulting behaviour of juvenile RKC in pods. Interestingly, he noted that all crabs moulted outside the pods at night, most of them shedding their exoskeletons between January and March. While it is known that juvenile RKC may undergo two moults per year (Stevens, 2014), it was unexpected to observe that some of the carbs in our experiment began the moulting process in October (Table 5). It should be noted that the trial period was not intended to coincide with moulting, as shorter photoperiods during fall months generally inhibit this process. Moreover, moulting has been shown to negatively impact meat content in RKC, as they reduce or cease feeding, and their newly grown exoskeleton occupies space previously occupied by meat (Stevens, 2014). Six crabs in this study underwent moulting, potentially skewing the results. Table 5 presents differences observed before, during, and after the trial, indicating that only the moulted crabs exhibited weight gain. Table 6 shows the difference in weight, carapace length (CL), and carapace width (CW) for both moulted and non-moulted crabs, demonstrating the negligible weight gain and no difference in CL and CW for non-moulted crabs. Although RKC gains muscle between moulting, this muscle growth replaces the free body fluids in the space

between exoskeleton and muscle. Despite their high feeding activity, this is evident by the minimal weight gain in non-moulted crabs (Table 6).

Both James et al. (2013) and Lian et al. (2021) observed thinner thickness of the leg exoskeleton in RKC after moulting, with observations of up to 65% thinner exoskeleton thickness in spring harvested RKC compared to RKC harvested during autumn. In this study it was significant differences between mean exoskeleton thickness of the moulted crabs compared to the non–moulted (Table 11). Our results coincide with the result of the other two studies, moulting influences exoskeleton thickness. Due to the thin and fragile exoskeleton, handling and fishing on the RKC during and after molting periods may harm the animal.

The hepatosomatic index (HSI) is a valuable indicator of the nutritional and biological status of RKC, as the hepatopancreas plays a crucial role in energy storage and metabolism. The HSI was found to be similar across all groups in Table 11, indicating that the nutritional status of the crabs did not differ significantly. RKCs require significant energy during molting and tend to reduce their feed intake or cease eating altogether, as depicted in Figure 8. This phenomenon is accompanied by the mobilization of energy reserves such as proteins, lipids, sugars, and other carbohydrates from the hepatopancreas to the new cuticle that is forming through the hemolymph. Therefore, differences in the HSI between moulted and un-moulted crabs are expected, as described by Lian et al. (2021). However, as shown in Table 11, no significant difference between the groups was observed in the HSI. This may be attributed to the fact that some of the moulted crabs completed the moult well before processing, thus allowing them to eat enough to balance out the expected differences. The diets did not significantly affect the HSI in this study, consistent with James et al. (2013) findings, which revealed no significant difference in HSI between four different diets. Lian et al. (2021) observed a higher value in the CI after molting than before molting. This study also showed that the CI between the diet groups, reference group, or moulted crabs did not differ. This may be due to the crabs need for the cheliped to ensure foraging, as they keep the muscle in the chelipeds throughout molting. Table 11 indicates that the CY of the crabs before cooking was similar across all groups. However, a significant difference was observed between moulted and non-moulted crabs after cooking. Lian et al. (2021) attributed this difference to the space between the exoskeleton and muscle, occupied by free body fluid lost upon cooking.

The samples were analysed for moisture, ash, fat content, and FA profiles of both cooked crab meat and raw hepatopancreas. The moisture, ash, and fat levels in the cooked crab meat were consistent with expected values and can be compared to other studies with similar outcomes, as Lian et al. (2021). The smaller crabs in the trial naturally had smaller hepatopancreases. The fat content analysis methods used by ALS Laboratory (NIR and GC-FID) may be sensitive to small sample amounts. Due to the size of the crabs in the feed study, it is likely that a good measurement could not be obtained. Nevertheless, the moisture, ash, and fat values in the crab meat and the moisture and ash values found in the raw hepatopancreas are consistent with the results of Lian et al. (2022); thus, the fat content in the hepatopancreas may also be similar. The moisture content was found to be approximately 80% in the leg meat of the RKC (Table 11),

while the moulted crabs showed higher variability, as expected, due to lower meat content and higher water content.

There were no significant differences found in the FA profile in cooked leg meat content, this may be due to the short duration of the study. However, significant differences were found in the hepatopancreas in the ∑MUFA and ∑PUFA. This may be due to the differences in FA profiles of lumpfish and the dry feed. The FA profile of farmed lumpfish was described by Ageeva et al. (2021). This FA profile differs from that of the dry feed used in the feed trial (Figure S2, Appendix). The FA profile of lumpfish will also differ from the diet fed to the reference crabs. However, sufficient data on the reference crabs, such as their feed intake, moulting, and diet, were not available. This makes it difficult to compare them with diet groups A-D. Nevertheless, it is noteworthy that RKC are individual animals with different behavioural traits that may affect the results. It is anticipated that longer experimental periods will reveal more substantial discrepancies in fatty acid profiles, particularly in the hepatopancreas.

The FA ratios are consistent with those reported by Lian et al. (2022); but the exact values differ. Comparable studies have shown different values for the FA profile, likely due to the use of wild RKC in these trials. Dvoretsky et al. (2022) stated that “you are what you eat” is a fitting statement when discussing the FA profile of the RKC. He saw that the FA profile changed if the crab foraged on soft or hard bottom, reflecting the crab's diet. These differences in diet may explain some of the variation seen between the results from this trial and other studies. The low fat content, but high percentage of n−3 FA indicate a good nutritional quality of the leg meat. Dvoretsky et al. (2021) observed that higher levels of PUFAs in the meat are consistent in marine crustaceans, which coincides with the results presented in Table 12. This finding is also consistent with the observation that, in crustaceans, muscles are the major site of protein storage, whereas fat is stored in the hepatopancreas, as discussed by Dvoretsky et al. (2021).

5 Conclusion

In this study, the most effective diets observed were dry feed coated with lumpfish hydrolysate and dry feed combined with lumpfish. In addition, the use of an attractant in the feed seems to stimulate the crab's appetite. These findings suggest that omnivorous species may benefit from diets consisting of multiple components and that lumpfish can increase the appetite of RKC. Notable individual differences in the feeding patterns of RKC were observed. It is important to be aware of these differences when upscaling similar experiments. Further research is necessary to fully understand both the nutritional requirements and feeding habits of these crabs. In 2019, 42 million lumpfish were used in the salmon aquaculture industry. These fish are often discarded as waste once they are no longer of use. Furthermore, the substantial quantities of juvenile RKC that are currently discarded as waste represent a valuable resource. By combining these two under-utilized resources, one can promote sustainable practices within the aquaculture industry. However, more research in optimizing the diet and feeding regimes is needed. In addition, infrastructure needs to be in place before a commercially viable industry can be established.

In the continuation of this study, 300 crabs will be fed using a cost-effective feeding approach that includes the lumpfish hydrolysate and lumpfish. Feeding with lumpfish will be complemented with dry feed to meet the nutritional requirements of the RKCs. By using "trash as treasure," we can move towards a more efficient and sustainable approach to resource utilization. Overall, this study provides important insights into the feeding habits of RCKs and may provide a basis for future research in sustainable feed production.

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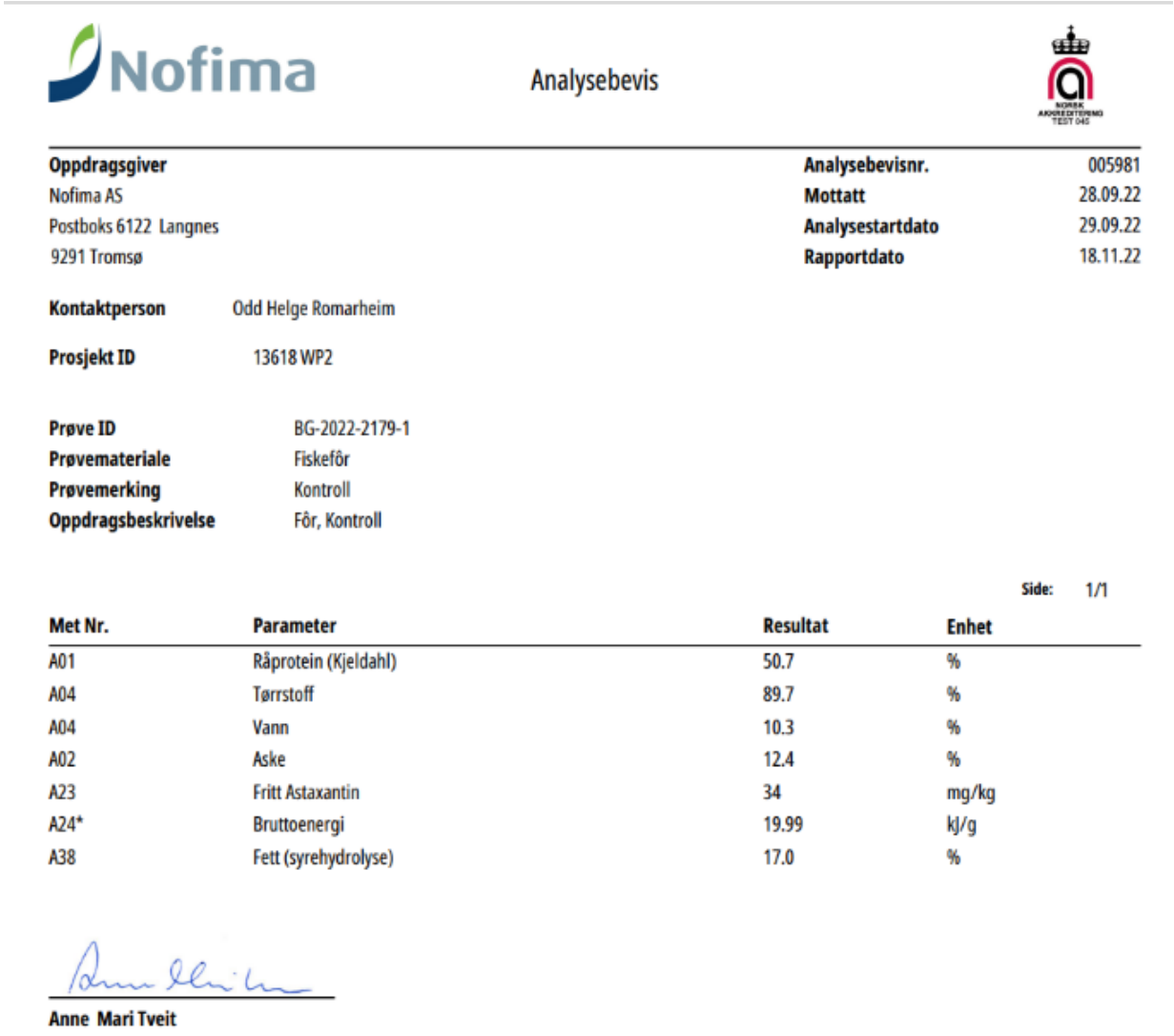
7 Appendix

Figure S1 illustrates the nutritional values in the dry feed used in the feed study.



Nofima

Analysebevis

Oppdragsgiver		**Analysebevisnr.**	005981
Nofima AS		**Mottatt**	28.09.22
Postboks 6122 Langnes		**Analysestartdato**	29.09.22
9291 Tromsø		**Rapportdato**	18.11.22
Kontaktperson	Odd Helge Romarheim		
Prosjekt ID	13618 WP2		
Prøve ID	BG-2022-2179-1		
Prøvemateriale	Fiskefôr		
Prøvemerking	Kontroll		
Oppdragsbeskrivelse	Fôr, Kontroll		

Side: 1/1

Met Nr.	Parameter	Resultat	Enhet
A01	Råprotein (Kjeldahl)	50.7	%
A04	Tørrstoff	89.7	%
A04	Vann	10.3	%
A02	Aske	12.4	%
A23	Fritt Astaxantin	34	mg/kg
A24*	Bruttoenergi	19.99	kJ/g
A38	Fett (syrehydrolyse)	17.0	%

Anne Mari Tveit

Figure S1 Nutritional value of the dry feed

Figure S2 illustrates the FA profile of the dry feed used in the feed study.

Prøve ID			BG-2022-2179-1
Prøvemerking			Kontroll
Prøvemateriale			Fiskefôr
Met nr.	**Parameter**	**Enhet**	
A56*	Fett (Bligh_Dyer)	%	17.7
A68	14:0	g/100g lipid	5.6
	16:0	g/100g lipid	12.2
	18:0	g/100g lipid	1.5
	20:0	g/100g lipid	0.1
	22:0	g/100g lipid	<0.1
	16:1n-7	g/100g lipid	3.5
	18:1 (n-9)+(n-7)+(n-5)	g/100g lipid	13.3
	20:1 (n-9)+(n-7)	g/100g lipid	8.7
	22:1 (n-11)+(n-9)+(n-7)	g/100g lipid	12.4
	24:1n-9	g/100g lipid	0.7
	16:2n-4	g/100g lipid	0.3
	16:3n-4	g/100g lipid	0.2
	18:2n-6	g/100g lipid	2.9
	18:3n-6	g/100g lipid	<0.1
	20:2n-6	g/100g lipid	0.2
	20:3n-6	g/100g lipid	<0.1
	20:4n-6 (ARA)	g/100g lipid	0.3
	22:4n-6	g/100g lipid	<0.1
	18:3n-3	g/100g lipid	1.2
	18:4n-3	g/100g lipid	1.8
	20:3n-3	g/100g lipid	<0.1
	20:4n-3	g/100g lipid	0.4
	20:5n-3 (EPA)	g/100g lipid	5.2
	21:5n-3	g/100g lipid	0.2
	22:5n-3	g/100g lipid	0.5
	22:6n-3 (DHA)	g/100g lipid	7.7
	Sum mettede fettsyrer	g/100g lipid	19.5
	Sum monoene fettsyrer	g/100g lipid	38.6
	Sum (n-6) fettsyrer	g/100g lipid	3.5
	Sum (n-3) fettsyrer	g/100g lipid	17.2
	Sum flerumettede fettsyrer	g/100g lipid	21.2
	Sum (EPA + DHA)	g/100g lipid	12.9
	Sum identifiserte fettsyrer	g/100g lipid	79.3
	Sum uidentifiserte fettsyrer	g/100g lipid	5.1
	(n-6)/(n-3)		0.2

Resultatene gjelder kun de analyserte prøver slik de ble mottatt. De kan ikke gjengis i utdrag uten vårt samtykke. Vennligst kontakt oss dersom analyseusikkerhet ønskes tilsendt.
Metodereferanser for akkrediterte analyser: https://nofima.no/fasilitet/biolab/
Stjerne * angir analyse som ikke omfattes av akkrediteringen.

Figure S2. FA profile of the dry feed.