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Development of Alternative Baits Based on Rest Raw Materials, Low-valued and Surplus Fish for Baited Gear Fishing of Atlantic Cod (*Gadus morhua*)

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Acknowledgments

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Ås, August 14th, 2018

Prajwal Pradhanang

Abstract

The experiment was conducted to develop the alternative baits from low-valued and surplus fish for pot and longline fishing of Atlantic cod. Minced herring and capelin were used to produce baits for pots with increasing level of alginate (2%, 4%, and 6%) and microbial transglutaminase (TG) (0.5%, 1.5% and 2.5%) as the binding agents to attain the optimal handling and water stable baits using a low binder. Alginate-based baits were shaped through stuffing technique while TG-based baits through molding and setting. Alginate supplementation at 4% of the total weight of the bait (4g/100g) gave good handling properties and satisfactory water stability which was easily processed. This level was used to make pot baits from the other two species of fish namely whole minced sprat and minced trimmings of saithe. Four types of baits (5 bait pieces per tank) were subjected to a behavioral study with cod (5 fishes per tank) in three experimental tanks to study their species preference over baits of different species. Response graphs from multivariate principal component analysis of different behavioral patterns showed that herring and sprat gave more attractive baits than capelin and saithe. Herring and capelin baits made with TG did not produce suitable baits. Although, it was compared with alginate-based baits of respective species to study the response difference of binders in cod. There was not observed a substantial difference in behavior of cod in response to the two binders in baits. Industry-processed skin from saithe was used for making baits for longlines, and compared with the baits made from the skin of manually deskinned herring and mackerel. Only the saithe skin was suitable for longline baits owing to its better hooking strength. Behavioral analysis of cod showed poor response to saithe skin bait when compared to highly preferred weak herring and mackerel skin baits. Improvement in taste factor of saithe skin bait was made by either incorporating Ecobait attractants (EA) (5% and 10%) or by coating saithe skin layer over highly preferred herring bait. Response graph and PCA biplot showed improvement in sensory characteristics of these baits compared to pure saithe skin baits. The breaking strength declined linearly with increase in EA concentration while skin coated bait gave the lowest value. All the improvised skin baits showed appreciable hooking stability.

Keywords: Atlantic cod, alternative bait, skin bait, pot fishing, longline fishing, alginate, transglutaminase, behavioral response, herring, capelin, sprat, saithe, mackerel.

Abbreviations

NOK	Norwegian kroner
LIFE	Low impacts and fuel efficient
NEAC	Northeast Arctic cod
H	Herring
C	Capelin
B	Sprat
S	Saithe
SS	Saithe skin
HS	Herring skin
MS	Mackerel skin
EA	Ecobait attractant
A	Alginate
TG	Transglutaminase
ANOVA	Analysis of variance
R	R statistical program
PCA	Principal component analysis
kg	kilogram
m	meter
cm	Centimeter
mm	millimeter
min	minute
h	hour
s	second

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1. Introduction

The Atlantic Ocean is the home to a wide habitation of Atlantic cod (*Gadus morhua*) on both sides of the ocean. But its economic importance as capture fishing is established immensely in the Northern part. Cod fisheries account for more than half of fish landings in Norway (FAO, n.d.; Heen et al., 2014). Fishing is done with several methods, and gill nets are commonly used. This method, however, is realized as an unsustainable method giving random and low-quality catch (Akse et al., 2013). Baited gears like pot and long-line fishing are more species-specific providing high-quality cod and high-valued products like stock-fish and clip-fish, making this a preferred way of fishing cod (Larsen, 2015; Olsen, 2014; Suuronen et al., 2012). The key element of the baited-gear system is bait which plays a crucial role in increasing catch specificity and hence decreasing bycatch. Fresh fish of high-quality is generally used as the bait which is bought at a high price as they are highly valued products used for human consumption (Løkkeborg et al., 2014). Furthermore, an increase in bait price from NOK 9 to NOK 15 per kg had elevated the operating cost of baited gear system by 10 % that demotivates the fisherman to explore fishing through baited-gear lines and pots (Carvajal et al., 2015). This calls for the development of alternative bait which is easily available at low price and contains acceptable properties of bait for cod fishing.

Utilization of waste and low valued marine products that are preferred by cod is the most economical and sustainable way of making baits. Norwegian fisheries and aquaculture produce large quantities of residual raw material from marine resources, and they have been sufficiently utilized on lands by different processing industries for the production of value-added products like feed, fish meal and oil, protein hydrolysates, cosmetic and pharmaceutical products, fertilizers, gas, and oil. Most of the residual materials from pelagic fish are used completely while only 30-40 % of white fish residues are used (Richardsen et al., 2015). However, there still exists a large gap in the supply chain for utilization of low values marine products especially the rest raw materials generated from fishing fleets and vessels. These materials usually constitute heads, bones, viscera, skin, and bycatches which accounts for more than 70% of total catch. Lack of space and high carrying cost impels them to dump most of least valued materials obtained after on-board processing on the sea (Jouvenot, 2015; Richardsen et al., 2015; Rustad et al., 2011). Development of simple technology that can easily be operated on-board utilizing their self-generated rest raw materials can have a great potential in delivering a cheap and consistent source of bait for cod fishing. Furthermore, low-valued by-products and surplus fish obtained during high capture season can be tapped as a valuable resource on the land for development of bait that is highly attractive to cod.

Few industries are producing alternative baits to natural one, sharing very little information about process technology. These industries are still in the phase of developing highly targeted bait for cod. There is also a limited number of studies conducted in laboratories and real field to understand the behavior of cod fed different baits. This paper provides new insight on the development of bait from different species of whole fish and trimmings for the pot and longline fishing of cod based on a study of their behaviors in response to the baits used in experimental tanks.

2. Objectives

The main objective of the thesis is to study the efficiency of baits for capture fishing of Atlantic cod by utilizing surplus fish and rest raw materials from the fish processing industries and fishing fleets. The thesis is specifically aimed at:

1. Development of bait with good handling strength and water stability achieved by using low binder content that must be used in pot fishing of cod.

2. Understanding the degree of preference of the alternative baits made from different fish species.

3. Development of strong skin bait based on fish skin that is highly preferred by cod.

4. Development of effective alternative ‘skin-coated bait’ for the longline fishing hooks.

3. Hypotheses

H1. Binders enhance the texture of the baits.

H1-1. Increase in binder content improves the handling and water stability of the bait.

H1-2. Different type of binders has differing binding effect in the bait.

H1. Cod has an unequal approach towards the alternative baits of different species.

H1-1. Cod have a high preference for the bait of their natural feed.

H1-2. Fish skin coating enhances the feeding perception of cod.

H1-3. Attractants-skin coating is preferred over pure skin coating.

H3. All types of skin baits are equally strong for fishing with longline hooks.

4. Literature Review

Since the project on fish-based alternative baits and the extruded-coated baits was conducted simultaneously between me and my teammate (Lifeng Zhao) in NMBU and Ecobait AS; we performed the shared review of the literature. This section particularly deals with the background information about the behavior of cod toward the natural bait used in a pot and longline fishing of Atlantic cod and development of artificial baits for cod.

4.1 Baited gear fishing of Atlantic cod (*Gadus morhua*)

Pot and longline fishing are Low Impacts, and Fuel Efficient (LIFE) baited gear capture techniques that are widely used for the fishing of Atlantic cod. They tend to have the least impact on the aquatic ecosystem with a high degree of species-selective fishing (Suuronen et al., 2012).

4.1.1 Baited cod pot fishing

Baited pots are an enclosed compartment of a metal frame with conical entrance and the bait inside to lure the fish equipped with holding chamber for fish (Hedgärde et al., 2016; Suuronen et al., 2012). It can be bottom set or floating type (Figure 1) with the later to avoid the bycatch of crabs (Furevik et al., 2008) and hinder seal interference (Ovegård et al., 2011). Pot fishing have relatively low capture efficiency; however, it has the advantage of being simple in construction, less use of energy with minimal habitat impact. Fishes delivered are live and are of high quality (Slack-Smith, 2001; Thomsen et al., 2010). The capture efficiency of the baited gear is primarily determined by the environmental variables such as water temperature, light levels, currents, density, an abundance of natural prey, the size distribution of competitors which influence the fish activity, feeding motivation and their sensory and locomotory abilities (Stoner, 2004). Besides, the catchability of pots is also affected by the designing of pots (pots shape and size, mesh size, entrance design) and especially the type of the baits (Hedgärde et al., 2016).

The capture principle of pot fishing is to attract and lure the fish inside the pot where it can be held until the pot is hauled. Bait plays an important role to attract the fish, especially for target species capture as fish are initially attracted by smelling the bait. Bait releases its natural attractants in the current creating an active space of odor plume in the concentration above the response threshold of the fish. However, the active space doesn't necessarily confirm for the response of all the fish within the space boundary towards the bait. Hence, catchability depends both on release rate and attractants transfer through the water and "chemosensory threshold for an individual fish at a specific time" (Furevik, 1994; He, 2010; Løkkeborg, 1998; Stoner, 2004).

Figure 1. Typical floating-type cod pot (Königson et al., 2015)

4.1.2 Longline fishing

Longline fishing gear consists of series of hooks connected to the main line through gangion and swivel at the hook spacing of around 1m or more (Figure 2). Baits are shot over the hooks through baiting machine and lines are set on the fishing ground (Larsen, 2015; Suuronen et al., 2012). The release of characteristics odor from the bait stimulate the fish to approach in its near vicinity and get hooked in assistance with the visual and textural stimulus. Hence, they are highly species selective capturing high-quality fish (Løkkeborg & Bjordal, 1992).

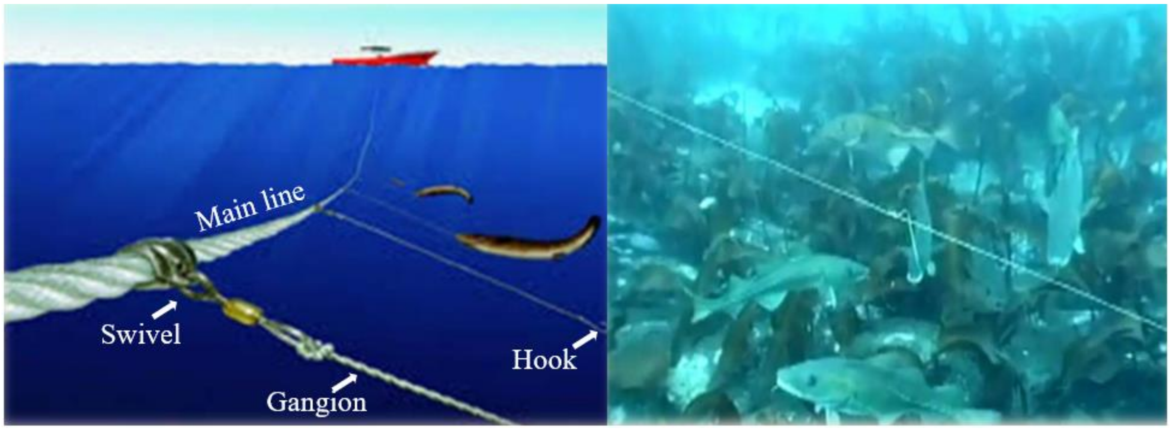


Figure 2. Longline fishing gear (left) and hooked cod (right) (Larsen, 2015)

4.2 Atlantic cod (*Gadus morhua*) feeding behavior and their food preference

Cod is an omnivorous fish and is opportunistic in their feeding habit. Juvenile cod primarily feed on crustaceans, small mollusks, and polychaetes while large cod are piscivorous feeding on large fishes and crabs. They can also exhibit cannibalism to some extent by feeding on their small ones. They localize prey using both of their visual and chemosensory mechanisms and exhibit diurnal variation in feeding being active at dusk and dawn while less active at night. They have a broad range of feeding depending on the abundance of prey that varies with seasonal and geographical differences. Catches through baited gears are high during their active feeding period while the abundance of natural prey leads to lower catchability. (Bogstad et al., 1994; Cohen et al., 1991; Daan, 1973; Løkkeborg & Fernö, 1999; Scott & Scott, 1988)

There exists a size relation between cod and prey in the food preference of cod over different prey species, which utilizes the energy efficiency of the larger prey species by large cod. From the stomach analysis of cod in the southern North Sea, Daan (1973) found shrimps and polychaetes to be the dominant food in the diet of small cod while clupeids (Sprat and Herring), flatfish (dabs and plaice), Portunus crabs and Aphrodite formed the bulk of the food eaten by large cod. Gadoids (mainly haddock) was the major part of the large cod diet in the Northern North Sea. In the northern areas of northeast US continental shelf, the cod diet consisted more of fish prey including herring, capelin, mackerel, squid, silver hake while southern areas tend to have crustaceans, mollusks and sand lance (Link & Garrison, 2002). Pachur and Horbowy (2013) observed clupeids as the dominant prey (more than 67% of total stomach content) of the cod in southern Baltic Sea. European sprats were found higher in medium size cod which was much higher in winter whereas herring was proportionally higher in larger cod. Other regularly fed diets were Gobiidae, Ammodytidae (sand lance and sand eel), young cod among the fishes and isopod *Saduri entomon*, shrimp *Crangon crangon* among crustaceans. Northeast artic cod (NEAC) are among the top predators in the Barents Sea that enormously feed on capelin. It forms an important part of the ecosystem as forage fish whose abundance has a significant effect on cod growth (Bogstad et al., 2015; Mehl & Sunnanå, 1991). Krill, amphipods, northern shrimp (*Pandalus borealis)* and capelin are the dominant prey species of small cod while the diet is immensely shifted to capelin as they grow large. Other important prey species are herring, polar cod, haddock, redfish and blue whiting (Dalpadado & Bogstad, 2004; Link et al., 2009). These NEA cod spending their lives in the offshore of Barents Sea migrate during spawning period towards the coastal areas all along the Norwegian coast and get overlap with local coastal cod which are almost non-migratory (Olsen et al., 2009). Michalsen et al. (2008) observed that they do feed during spawning on herring and Norway pout as their dominant prey species. Capelin is also the primary prey species of the cod in Icelandic waters and Labrador Sea (Lilly, 1987; Pálsson, 1983).

4.3 Preassessment principles of bait development

The success of the bait is known ultimately from the result of the field test, but it appears to be uneconomical for the online testing of every bait during the process of new bait development. Hence, a systematic assessment is needed to understand the potential of baits before field testing. The bait should have the basic characteristics of having potent attractants that can release for the considerable period to stimulate the fish to approach near and get trapped. This can be predicted in three steps according to (Daniel & Bayer, 1989)

- By analysis of soluble amines that can act as a potent attractant,
- By analysis of the release rate patterns of attractants and
- By behavioral assay of fish in tanks.

4.5 Behavioral study of cod with regards to baited fishing gear

Fish exhibits a specific behavioral pattern for a certain type of baits or attractants. Understanding the behavior of fish is therefore important for the successful fishing from a commercial baited gear. It is the preliminary step to be studied during the process of baited gear development, the findings from which can help build the foundation of research framework and explain the results obtained from the real fishing ground. Study of the fish behavior can be performed in different levels of investigation ranging from simple laboratory method done in captive fish to the more realistic full-scale fishing trials. Analysis of video filming of fish behavior in a closed tank can provide every small detail of individual fish response to the given bait. However, the study might be biased due to stimulation of fish by activity is done in their proximity during experiment owing to the limited volume of water. On the long run, fish might also adapt to the laboratory conditions showing behavior inconsistent with the actual fishing state. This problem can be solved by experimenting in the restricted field, but one should compromise with the variation in the degree of the response obtained due to inconsistent fish density and activity rhythms. The success of the bait can be broadly understood in the trial with baited gear fishing in the actual field or to a much higher level of study with full-scale fishing trial during the whole season demanding a vigorous effort and plan. However, one is yet fully unsure due to various factors coming into action in a real field that might result in inconsistency in repetitive trials. However, the value of behavioral study cannot be well explained in commercial fishing trial, the result obtained from laboratory response might well correlate to generate the holistic picture (Fernö & Huse, 1983; Løkkeborg et al., 1993). It is also shown by the number of research works that the value of behavioral study obtained from the laboratory does have reliability to the value of subsequent field trial especially with response concerning palatability, texture, and appearance of the bait but less related to the approach and taste response of cod towards bait studied between the tank and field (Løkkeborg et al., 1993).

Johannessen et al. (1993) categorized the behavior pattern of cod with regards to hooked bait into three phases: attacking, handling and terminating phase which consists of some behavioral sequences within the phase. The perception of bait smell stimulates the cod to approach near it and taste it by touching the bait with lips or barbel or bite it partially or completely. It is either spit out or chewed and jerk off with the bait in the mouth. The moment is terminated when they pull back and gets hooked.

4.6 Natural baits used in baited gears for cod fishing

Several natural bait species like squid, herring, mackerel, sauri, crab, shrimps are used in baited gears for cod fishing. These baits are widely used in pot fishing in their natural form. They are minced or cut for better attractant release, and frozen baits are kept inside the fine-mesh bags or perforated box which hangs inside the pot trap to lure the cod. Several studies have been conducted to improve the fishing of cod by pot traps focusing mostly on the design improvement of the cage for the successful retainment of cod in the cage. While only a few studies explain the efficiency of different baits to lure fish in the pot traps. Furevik and Løkkeborg (1994) investigated the cod catching efficiency of different baits in pot traps soaked for a day at the depths of 10-500 m along the north-western coast of Norway. They observed the highest preference of squid (*Illex* sp.) by cod over other baits. It was also observed that the cod have a higher preference of crab (*Cancer pagurus*) against herring (*Clupea harengus*) bait. In contrast, Ljungberg (2007) observed high catchment of cod in the pot baited with herring cuts in the Baltic Sea, south-east of Sweden while squid gave the low catch similar to the non-baited pots. This relates to the inexperience of cod over squid that is commonly not found there. Shrimp is also the potential bait giving high rates of catches in the Baltic Sea.

In longline fishing, both the attractant quality and strength of the natural bait is crucial for successful hooking of the cod. Generally, squid bait is preferred in longlines owing to be both the potent attractant for cod as well as its strength that can retain in the hooks and not readily detached from cod attack. Johnstone and Hawkins (1981) studied the performance of different baits; squid, mussel, mackerel and salted herring in the longline hooks in the sea of Scotland and found squid, mussel, and mackerel as a potent bait for cod while salted herring was least effective. These prey species for cod are commonly cut into pieces and hooked, or they might be minced and put in fine mesh bags and hooked for longline fishing. Løkkeborg (1991) performed the comparative study of squid bait pieces as a control against same bait kept inside nylon bag in the longline hooks in the coastal area of northern Norway and observed lower catch rate of cod owing to the negative effect of the bags. Also, minced herring with 4% guar gum enclosed in bag gave lower catch than control bait studied in the same trial. This study was done with an effort to utilize the surplus fish and replace traditional baits that are also used for human food with high bait price. Several works are being done to value-add underutilize marine resources including wastes from the fish processing industry and fishing vessels (Løkkeborg et al., 2014). The baits made from these products are often termed as alternative baits or artificial baits based on marine resources.

4.7 Development of alternative baits

Since the purpose of bait in fishing gear is to attract the fish from the far distance, 'attractant' forms the principal component of bait that elicit the food search behavior of fish. These stimulatory compounds are mainly amino acids such as alanine, cysteine, serine, glutamine, glycine, proline that seems to act individually or in combinations (Kasumyan, 1997; Raubenheimer et al., 2010). The source of attractant for alternative baits can, therefore, be from either a natural resource (surplus and waste products) or their hydrolyzed extracts and synthetic stimulatory compounds targeted for the species-specific capture. Protein hydrolysates of shrimp and blue mussels obtained from industrial waste were observed to be potential attractants for cod while capelin attractant was not effective enough to stimulate cod and be used in making alternative baits (Siikavuopio et al., 2017). The other component of bait is a binder that gives texture and consistency to the bait while it has its major role in the controlled release of attractants for a long period to somewhat mimic natural bait. Normally, when the bait is put is water, there is a high rate of release in the initial hour and decrease rapidly with the almost constant release in the later period. The prolonged release of attractants is believed to have high catchment of cod (Løkkeborg, 1990). While only the binder is not enough to provide shape and strength to the bait especially on longline fishing, it requires support or reinforcement that has been tried to achieve through various techniques such as use of bait bags and fiber matrix; surface hard gel coating of baits; high temperature-pressure extrusion or extrusion trough fiber mesh tubes (Løkkeborg et al., 2014).

Several efforts have been made to develop alternative baits, and many of them are patented giving little information about the composition and technology of bait development. Norbait DA, a Norwegian company, produces baits from fish wastes and offal using alginate as a binder or gelling agent. The minced materials are blended with a binder and extruded into a fiber mesh tube (cotton stocking) to give strength for easy handling in the baiting machine and improved hooking in the longlines (Norbait, n.d.) as shown in Figure 3. They claim to have online production of baits in the fishing vessels using their fishing byproducts with less preparation time, fast and clean baiting, reduced wastage and higher catch rates. However, the performance of baits against cod catchment is far less than other fish species (Løkkeborg et al., 2014). Beside species selectivity, this might relate to the negative effect of fiber matrix or decrease in freshness due to a high degree of thawing and mincing as cod prefer to feed on live prey.

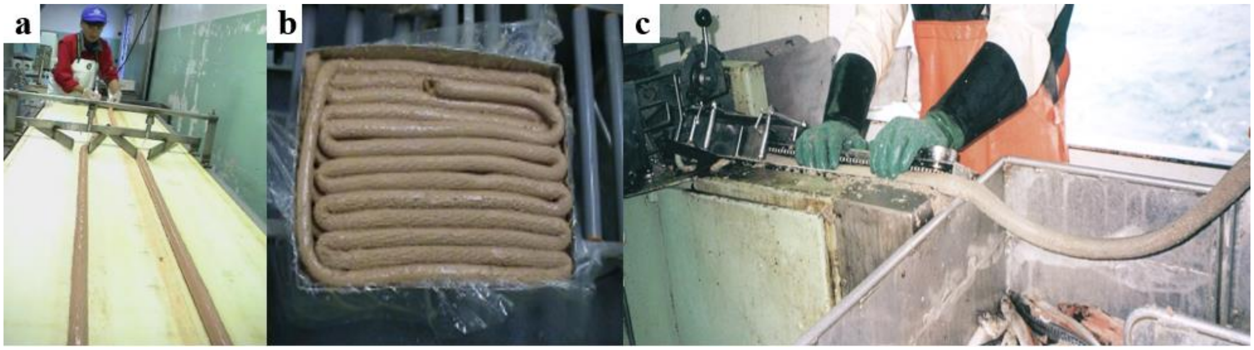


Figure 3. Norbait (a) Processing of bait (b) frozen baits in the box (c) baits passing through baiting machine (Norbait, n.d.)

Studies have been done in Iceland in collaboration with other institutes and companies in Spain and Portugal under the EU project regarding the manufacture of artificial fish baits based on fish waste named by 'artibait' (Tryggvadóttir et al., 2002). They used capelin, sand eel, squid and fish waste from processing plants and shellfish factories as raw materials for the development of artificial bait focusing especially on the freshness of the baits. They invented the method to avoid thawing, mincing and stirring that spoils the freshness characteristics of the bait. Raw materials are grated directly in their frozen state and kept in fiber bags which softens giving skin texture when wetted in water (Figure 4). The method is termed as 'snow technology' and has been patented (Morgunbladid, 2004). The Icelandic company Bernskan ehf (Sudavik Iceland) produced baits by applying high pressure on frozen raw materials and fish waste and injected into the cellulose fiber bags. Besides having several handling advantages of bait bags, they produce poor catch rate of cod in the Norwegian field (Henriksen, 2009).

Figure 4. Artibait made from grated frozen materials (left) in fiber bags (right) (Tryggvadóttir & Vala, 2005)

5. Materials and Methods

5.1 Production of baits

Production of baits was performed in Ecobait AS, Måløy. The experiment was carried out in April and May 2018.

Four different kinds of fishes namely herring (H), capelin (C), sprat (B) and saithe waste (S) were used as raw material for the experiment (Figure 5). Herring (*Clupea harengus*, Norwegian - sild) and sprat (*Sprattus sprattus*, Norwegian - brisling) were obtained from Pelagia Kalvåg, Norway. Capelin (*Mallotus villosus*, Norwegian - lodde) was obtained from Nils Sperre AS, Norway and Saithe (*Pollachius virens*, Norwegian - Sei) trimmings and skin were obtained from Måløy Seafood, Norway. They were ground through the toothed grinder (Figure 6) and stored frozen at -20 ºC until use.

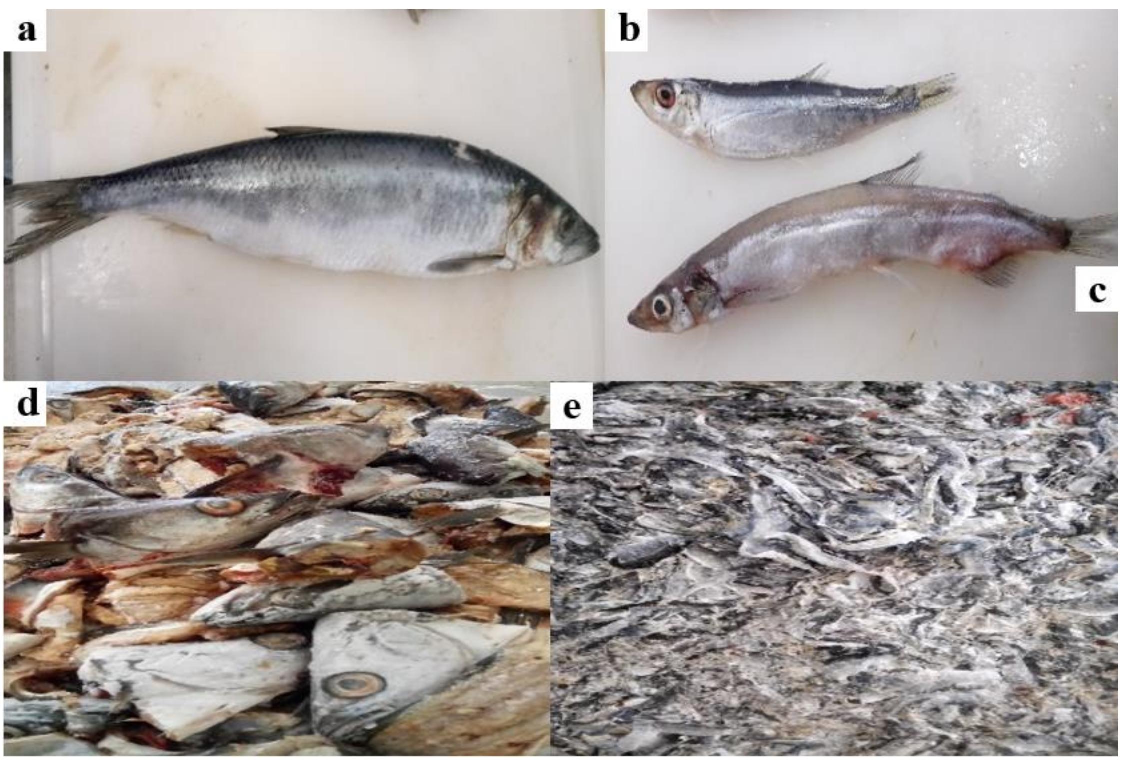


Figure 5. (a) Herring; (b) Sprat; (c) Capelin; (d) Saithe trimmings; (e) Saithe skin

Figure 6. Grinder

The experiment aimed at making baits for fishing of Atlantic cod in pot traps and longline fishing hooks from raw materials received in Ecobait AS, Måløy. This required several pre-trials to be done before the appropriate experimental design was made, as discussed in this section.

5.1.1 Pre-trials

Pre-trials were conducted with the herring, primarily to get the sense of minced fish properties that can be optimally stuffed. The equipment used for mincing and grinding is shown in Figure 7. Three variations of particle size were stuffed at constant binder level. In the first trial, ground herring was blended with 4% of alginate powder for a minute and directly stuffed to obtain a sausage. The other two were minced through two different mincer elements of die diameter 7mm and 3mm and then stuffed through a stuffer after blending with 4% alginate. The baits obtained from the trial were named as un-minced bait, coarsely minced bait, and finely minced bait respectively. The overall process is simplified in Figure 8. Selection of a mincer element for conducting further experiment was based on simple observation of the bait considering both the bait strength and economic aspect of the processing. It was observed that fine mincing (3mm) gave a uniform bait with good strength and handling properties while un-minced bait was easily breakable and hence rejected. Although coarsely minced (7mm) bait was slightly less scored than finely minced regarding strength, uniformity, and handling, the mincing process was smooth through 7mm mincer die hole. There was frequent stuck of tiny bones while running through 3mm mincer hole which decreases the process efficiency in the long run. Considering this, coarse mincer (7mm die diameter) was selected for mincing for the actual experiment.

Figure 7. (a) Mincing elements; (b) blender; (c) grinder; (d) mincing and grinding device

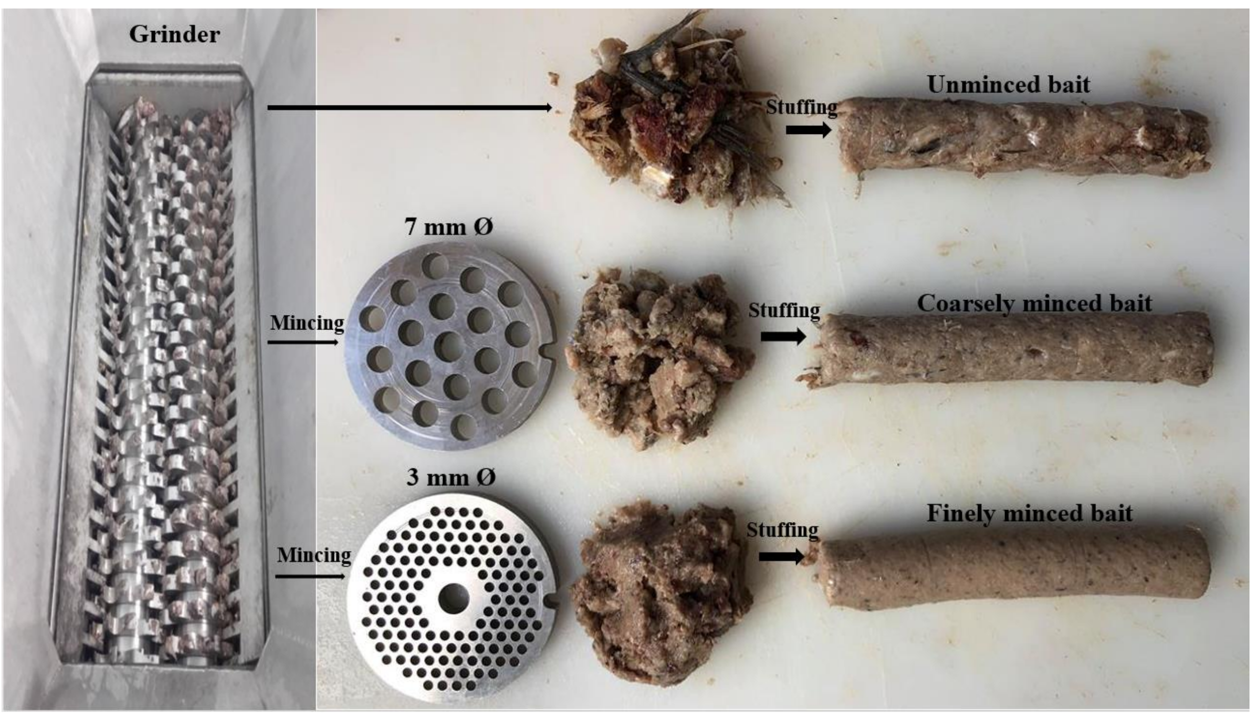


Figure 8. Simplified process flow from grinding to stuffing showing three variations of mincing

A pretrial was also done to set the limit of binder level for constructing the experimental design. Coarsely minced herring was blended with 1% and 10 % alginate powder and stuffed to understand the characteristics of the binder with minced fish and trimmings. It was observed that the bait made from 1% alginate was too weak to handle while 10% alginate bait was too hard. Another trial was also done by blending with 0.1% and 5% microbial transglutaminase (TG). The use of TG (Activa EB transglutaminase products, Ajinomoto), being the enzymatic binder, did not provide sufficient immediate binding, as it requires warm temperature and a long time to react with meat particles and set. Thus, the minced herring after blending with TG was molded in hemispherical cups and set at 30 ºC for overnight. Weak bait was observed at 0.1% TG and hard crust bait at 5% TG. This gave an idea to construct the experimental design within a suitable limit of binder level which is discussed further in the paper.

5.1.2 Experiment work

The experimental work was divided into four phases. The first two phases focused on the development of bait for pot fishing and the last two phases for longline fishing.

5.1.2.1 First phase (Bait Group 1)

In the first phase of the work, frozen ground herring and capelin were thawed for an hour in ambient condition and minced through a household mincer (Kenwood) of 7 mm mincer hole in the partially frozen state. This was done to maintain the freshness of the fish. The minced product was then blended with varying proportions of alginate (A) and transglutaminase (TG) binders respectively as shown in Table 1 marked with respective codes. This matrix was grouped as Bait Group 1.

Table 1. Bait Group 1

Species	Binders (%) w/w					
	Alginate (A)			Transglutaminase (TG)		
	2	**4**	**6**	**0.5**	**1.5**	**2.5**
Herring (H)	HA2	HA4	HA6	HTG0.5	HTG1.5	HTG2.5
Capelin (C)	CA2	CA4	CA6	CTG0.5	CTG1.5	CTG2.5

Alginate-based binders were stuffed through the stuffer of 3 cm diameter and instantly frozen at -20 ºC (Figure 9a & 9b). TG-based binders were kept in the mold (hemisphere cup, 2.5cm radius) and left overnight to set at 30 ºC (Figure 9c & 9d). Effect of two different binders and their level on the physical properties of bait were studied by analyzing the moisture content, water activity and water stability of the bait.

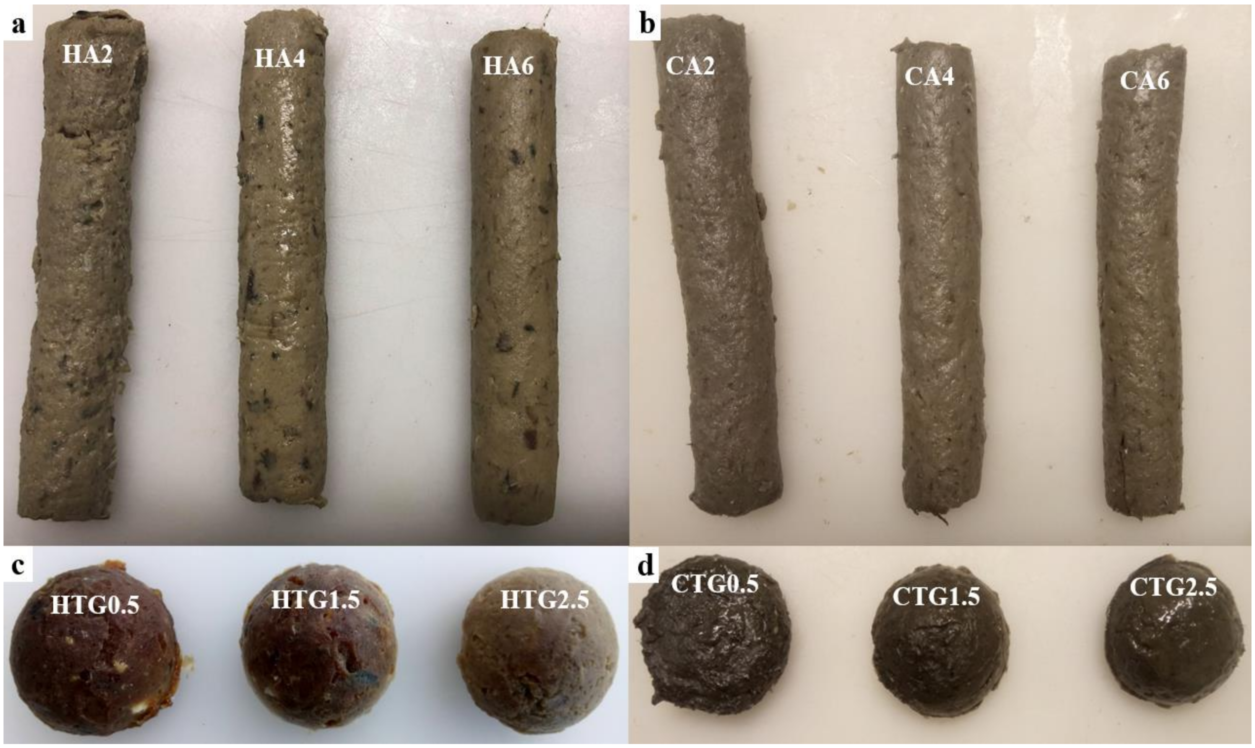


Figure 9. Bait Group 1 (a) Herring-alginate baits; (b) Capelin-alginate baits; (c) Herring-TG baits; (d) Capelin-TG baits

5.1.2.2 Second phase (Bait Group 2)

Frozen ground sprat and saithe trimmings after thawing were similarly minced and blended with 4% alginate followed by stuffing and freezing at -20 ºC to produce the baits: BA4 and SA4 respectively. These baits along with herring and capelin bait pooled from 'Bait Group 1' containing 4% alginate and 2.5% TG were grouped as 'Bait Group 2' to study the response of cod towards four different species and two different binders of the same species (in herring and capelin) (Figure 10). The response design is simplified in Table 2.

Table 2. Bait Group 2

Cod response to species		Cod response to binders	
	Herring	HA4	HTG2.5
	Capelin	CA4	CTG2.5
	Sprat	BA4	
	Saithe	SA4	

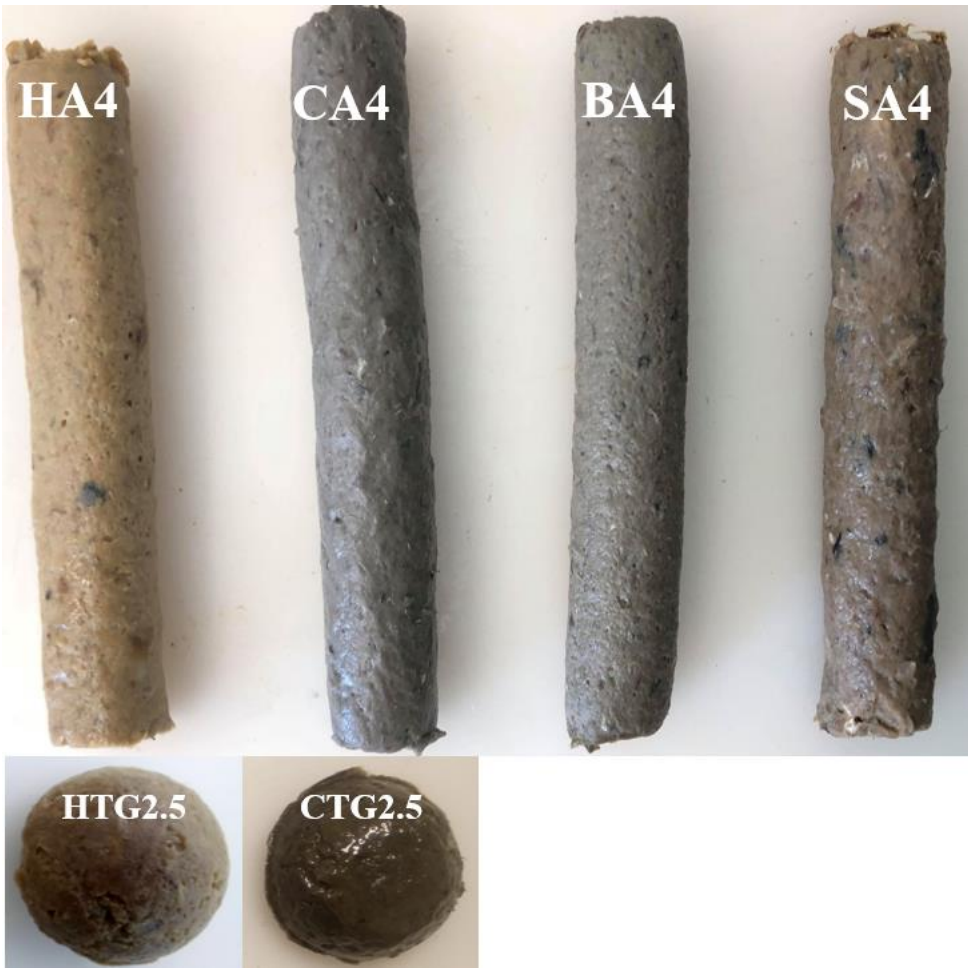


Figure 10. Bait Group 2

5.1.2.3 Third phase (Preparation of fish skin gel)

A pre-trial was done to find the optimum cooking time of fish skin. Saithe skin was added with 30% water and heated at 60ºC at five mins intervals from 10 to 30 mins with continuous stirring.

Warm skin solution was kept in the mold (hemispherical cup) and let it set overnight at cool temperature ca. 5ºC. The gel was set well at the minimal time of 10 mins cooking. This cooking process was selected for a further experiment with skin.

Herring and mackerel were deskinned manually. Saithe skin, herring skin, and mackerel skin were cooked at 60ºC for 10 min in a water bath with continuous stirring and kept in the mold to set it for overnight as shown in Figure 11. They were grouped as 'Bait Group 3' (Table 3)

Table 3. Bait Group 3

Skin	**Code**
Herring	HS
Mackerel	MS
Saithe	SS

Figure 11. Bait group 3

Cod were filmed in the tanks to study the response of the fish towards the different species of skin baits. Herring and mackerel skin baits were readily disintegrable in contrast to tough saithe skin bait, so the only baits produced with saithe skin were subjected to breaking strength analysis.

5.1.2.4 Fourth phase

The final phase of the work aimed for the development of highly preferred and efficient strong bait used in the longline hooks for cod fishing.

Saithe skin was mixed with 5 and 10% attractant (Ecobait attractant) after cooking and left to set in the mold for overnight (Figure 12a). Table 4 shows the sample codes indicating the varying proportion of saithe skin (SS) and Ecobait attractant (EA).

Table 4. Saithe skin bait with 0, 5 and 10 % of Ecobait attractant (EA)

EA (%) (w/w)	0	5	10
Sample Code	SS	SSEA5	SSEA10

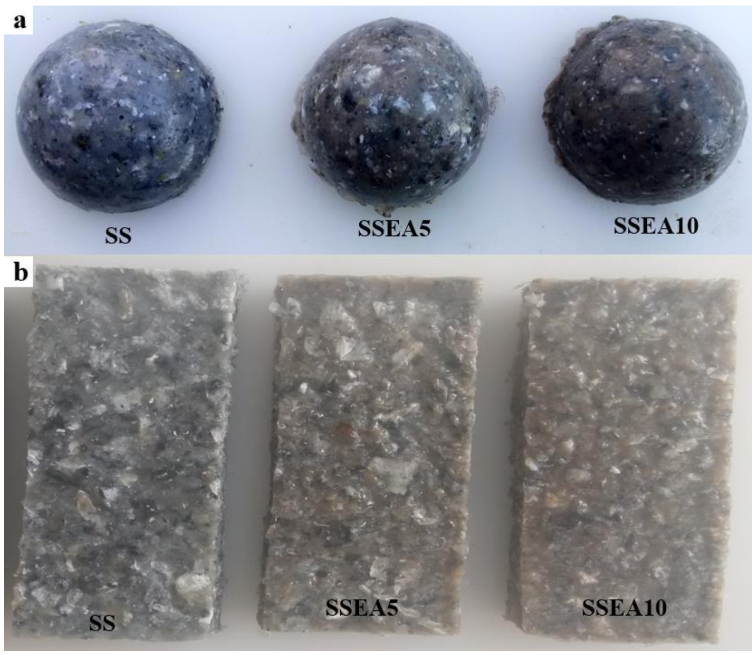


Figure 12. (a) Saithe skin bait molded in cups (b) Saithe skin bait set in a tray

These baits were subjected to cod response analysis by video filming in the tanks and hooking stability test.

For strength analysis, the cooked skin was set for a night in a thin tray of 1 cm thickness (Figure 12b), and breaking strength was measured by simple weight measuring device.

Saithe skin was also used as the coating layer over the highly preferred bait pooled from 'Bait Group 2'. The warm solution of cooked skin was ground in a mixer-grinder for a minute to make a coating solution as shown in Figure 13.

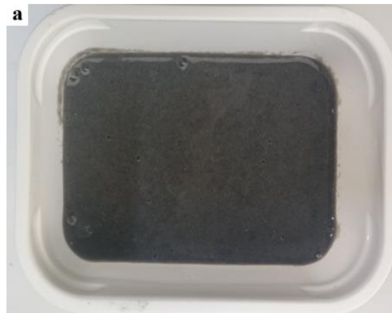


Figure 13. The coating solution of saithe skin

10 cm of HA4 bait was dipped in warm skin solution for a minute and dried in the cooler (ca. 5ºC) for 2 hours. The dried coated baits were again dipped and dried in cooler for overnight. The bait was named as SScoat (Figure 14). They were subjected to cod behavioral response, hooking stability and breaking strength.

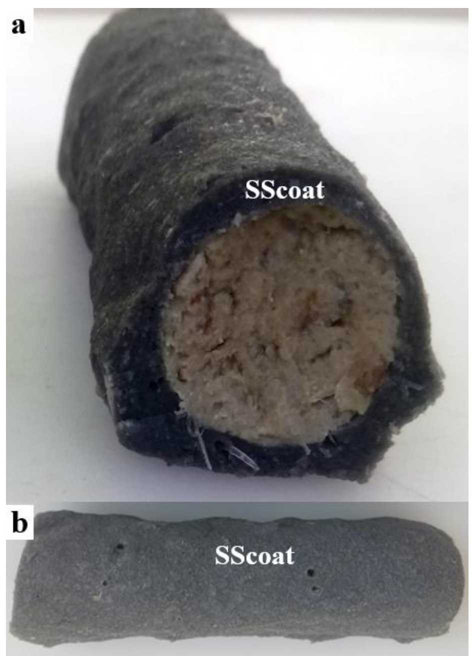


Figure 14. Saithe skin-coated bait (a) cross-sectional view; (b) lateral view

Bait made in the fourth phase were grouped as ‘Bait Group 4’ (Table 5)

Table 5. Bait Group 4

SS	SSEA5	SSEA10	SScoat

5.2 Analytical methods

Baits of 'Bait Group 1' were transported to NMBU to study the physical properties of baits such as moisture content, water activity, and water stability.

5.2.1 Moisture content

Moisture content was analyzed by hot air oven method (Tryggvadóttir et al., 2002). 10g of the sample was weighed on the aluminum moisture pan and dried in an oven at 103ºC for four hours. The dry matter was weighed, and moisture content of the bait was calculated as a wet basis.

Moisture content (%) = $((W_1 - W_2) / W_1) \times 100$

Where, W_1 = weight (g) of the sample before drying

W_2 =weight (g) of the sample after drying

5.2.2 Water Activity (a_w)

The water activity of the samples was measured using Rotronic hygrolab water activity measuring device (Appendix 10.7).

5.2.3 Water stability

Two different approaches to water stability testing of the baits were performed. The first approach was performed in the real field of the pot used in trap fishing. Perforated cylindrical box (Appendix 10.8) was used to observe the water stability of bait for 24 hours in open sea at a depth of 5m in Måløy fjord. The average seawater temperature (7 ºC) and water current (8 cm/s) on the analysis day was retrieved from yr.no.

The second was lab-based approach done in the shaker containing water that was set at the constant shaking frequency (Baeverfjord et al., 2006). Around 10 g of bait (3cm long for alginate-based bait and half-cut bait for TG-based bait) was weighed on a pre-weighed circular wire netting basket (20 cm long, 7 cm diameter, 3 mm mesh size, flat bottom situated 2cm above the lower end of the basket). Basket with bait sample was then placed in a 600ml beaker containing 300 ml water and fitted inside the water bath (Julabo SW22) at 22 ºC for two hours at 120 shaking per minute (Appendix 10.9). After incubation, the basket was dried in hot air oven at 103 ºC for 18 hours and weighed to determine residual dry matter present in the basket.

5.2.4 Handling properties

Baits with appropriate handling properties were selected based on simple observations (appearance and touching) during stuffing, shifting, packing, freezing, recovering, thawing and placing in pots that could be achievable with a low level of binder content. They were chosen to study the behavioral response of cod in the tanks situated in the study field.

5.2.5 Breaking strength

The breaking strength of skin bait was measured using a simple weight measuring device. Skin layer of 1cm thickness was clamped on one side and hooked at the other end connected to the measuring device. A constant force was applied until the layer was broken. The maximum force at the breaking point was noted as breaking strength of the bait. Skin-coated bait was hooked radially for measuring the breaking strength of bait. All baits were thawed for an hour before the test was performed.

5.2.6 Hooking stability

Hooking stability of 'Bait Group 4' was done by hooking the baits in the tanks with constant water flow (6 m^3/h) to the simulate realistic condition of the longline hooking activities (Appendix 10.10). The frozen baits were thawed for an hour before hooking. Hemispherical skin baits were hooked as it, while skin-coated bait was cut into 3 cm length. The difference in baits was observed after 12 hours of the test.

5.2.7 Video Filming of cod response

Filming of the baits was done to observe the perception-behavior of the cod for the bait in three tanks containing five fishes per tank (Appendix 10.11).

Circular fiberglass tanks with a diameter of 3 m were designed to contain the flowing seawater maintained at a depth of 0.9 m in the tank. Seawater from the nearby fjord was pumped through a pipe which divided the flow of water into three respective tanks. The inlet pipe in the tank was just below the water surface and bent tangentially to the wall of the tank creating a circular water flow. The outlet water flows through the bottom screen of the tank back to the sea. A GoPro camera was fitted in a movable stand to record a video for subsequent behavioral analysis.

Five baits per tank were dropped carefully in the middle of the tank just over the surface of the water to avoid splashing. Response tests with the baits were performed on every alternate day from the beginning of the week, i.e., Monday. The fishes were fed with mackerel cuts on Friday, leaving them without feeding during the weekend. The temperature of seawater (7 ± 2ºC) and time of filming was noted during each test.

The response of cod towards the bait was given a numerical score based on specific behavior acted on the bait. A complex set of behavioral sequence and interaction between the fishes when they were fed complicated the analysis of response towards the bait. Thus, for the sake of analytical simplification, the behavior pattern of cod was categorized into five actions, and overall response towards each bait was noted by analyzing the video. The five behavioral responses were termed as 'Touch', 'Nibble', 'Bite and spit out', 'Bite and eat slowly' and 'Instant swallow', and numerical score was given as the number of baits responded with the specific behavior shown by cod towards baits out of total bait fed i.e. 5 per tank. These responses sequentially explain the degree of preference of the bait by the cod.

5.3 Statistical Analysis

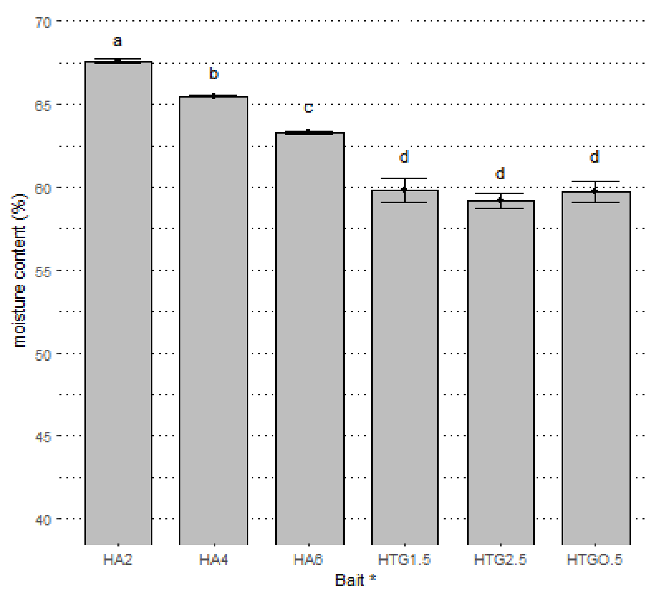
Statistical analysis was performed in R (version 3.5.1). One-way Analysis of Variance (ANOVA) was used to determine the effect of binder on moisture content, water activity and water stability of the baits. The breaking strength data of skin baits were also subjected to ANOVA. Tukey's HSD post hoc test was conducted for pairwise comparison of mean value between the baits. Results were presented as the mean ± standard deviation (SD). Differences were considered significant at $P < 0.05$. The response scores (as a number of bait) of specific behavior were compared between the baits as the proportional value of total baits fed to all three tanks treated as a single tank and the result displayed combinedly for each of the five behavioral actions. The proportional values of baits responded were pooled to conduct principal component analysis to study the main trend in the behavioral scores among them. The PCA biplots were shown to illustrate similarities and dissimilarities of cod behavioral patterns among different types of baits.

6. Results and Discussion

6.1 Bait Group 1

6.1.1 Moisture content

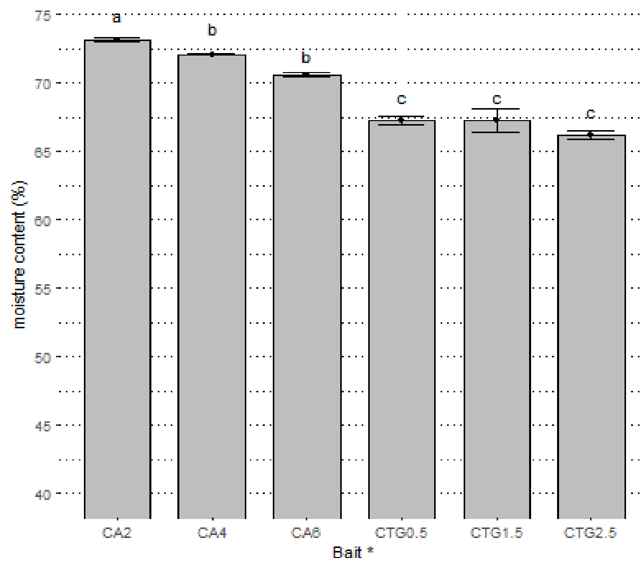
The moisture content of the 'Bait group 1' for herring and capelin bait was analyzed to study the effect of binder addition on the water content of the baits. Figure 15 shows the effect of alginate and TG addition on the moisture content of herring bait. Herring bait containing 2% of alginate (HA2) had the highest content of moisture (67.62 ± 0.27 %) which decreased proportionally and significantly with the increase in alginate content ($p < 0.05$). This can be understood as the additional effect of a binder containing high dry matter content. The moisture content of TG-based herring bait was significantly lower than alginate-based bait depicting the effect of a night long drying of herring-TG baits. However, increase in TG did not exhibit a similar reducing effect in the moisture content of herring bait. This might relate to the small incremental level of TG powder in the baits or due to inconsistency in temperature (30 ± 5 ºC) during the setting period in the warm cabin as bait was not made on the same day due to lack of enough mold cups.



***** Figure shows herring baits (H) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 15. Moisture content (%) of alginate and TG-based herring baits

Similar characteristics were observed in capelin baits (Figure 16). CA2 had the highest moisture content (73.14 ± 0.28 %) and reduce with the addition of alginate powder, though only CA2 and CA6 baits were significantly different among alginate-based baits. All TG based baits were significantly lower than alginate-based baits.

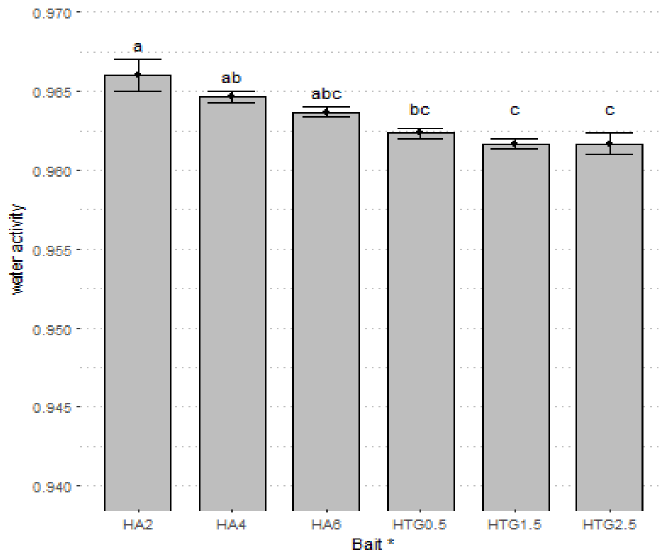


***** Figure shows capelin baits (H) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 16. Moisture content (%) of alginate and TG-based capelin baits

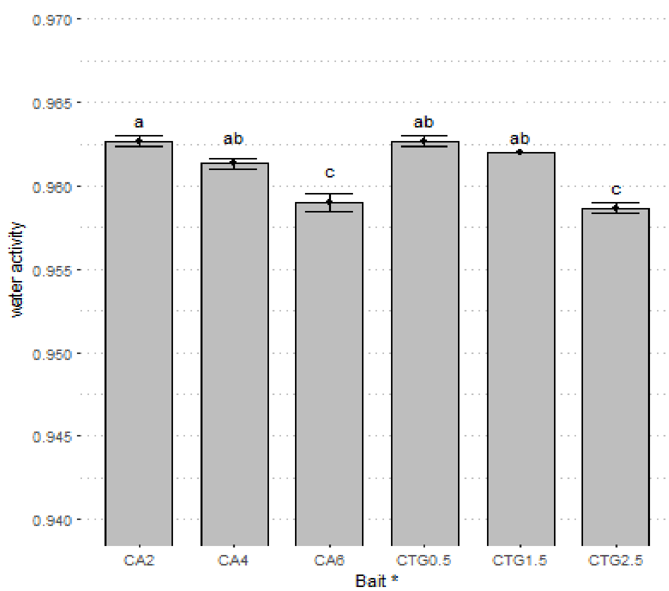
6.1.2. Water activity (a_w)

The very small difference in water activity was observed with the addition of binders. Water activity ranged from 0.962 to 0.964 in herring baits and 0.959 to 0.963 in capelin baits, the lower value being for 2.5% TG-based baits and higher value for 2% alginate-based baits in both of the bait species (Figure 17 and 18). Presence of high moisture might have masked the effect on binders in binding the free water. Huang and Clarke (2017) also did not find any significant difference in the water activity of tilapia fish balls made with different types of the binder.



***** Figure shows herring baits (H) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 17. Water activity of alginate and TG-based herring baits

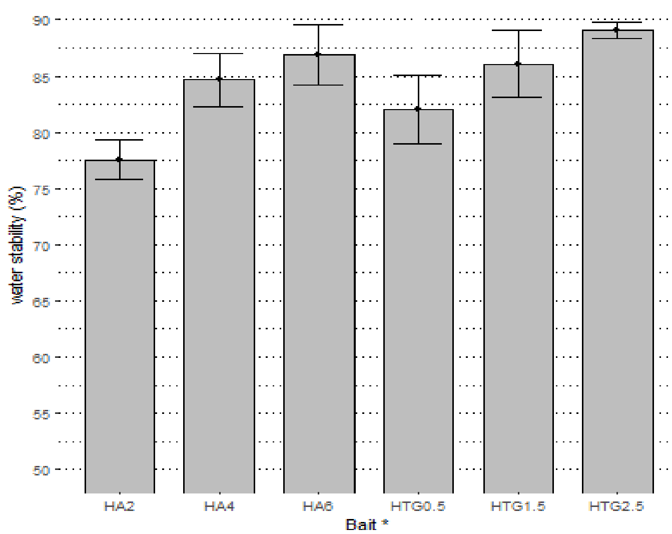


***** Figure shows capelin baits (C) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 18. Water activity of alginate and TG- based capelin baits

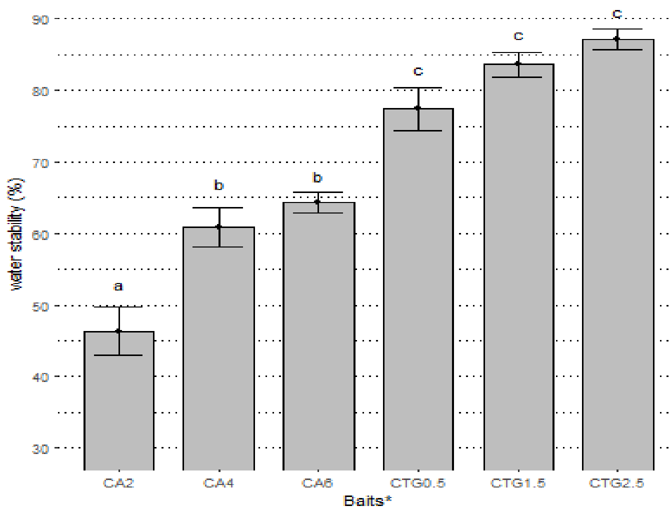
6.1.3 Water stability

All the baits including herring and capelin baits showed only a slight swelling without any disintegration in appearance after putting in pots in the sea for 24 hours. Experimental setting near the coastal fjord might contain low water current that didn't exert enough effect (as in the real field) on the baits placed in the pots. So, water stability test was done on lab at 120 shaking per minute that measured the dry matter content left in the pot after 2 hours. Though there was an increase in the mean value of water stability with an increase in binder proportion in herring baits (Figure 19), the difference was not significant due to the large variation observed within the sample during the experiment. Among capelin baits, CA2 had the lowest water stability than all other baits (Figure 20). TG-based baits were observed with slightly higher mean value than alginate-based baits which were more pronounced (significantly different) in capelin baits. TG being the enzymatic binder catalyzes the covalent crosslink between proteins (ε-(γ-glutamyl) lysine dipeptide) at the molecular level that might lead to lesser loss of dry matter into the water during shaking in the bath (Kieliszek & Misiewicz, 2014).



***** Figure shows herring baits (H) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 19. Water stability (%) of alginate and TG-based herring baits



***** Figure shows capelin baits (C) based on either alginate (A) or transglutaminase (TG) binder with different percentage of inclusion indicated by their respective number alongside. Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 20. Water stability (%) of alginate and TG- based capelin baits

6.1.4 Handling properties of baits

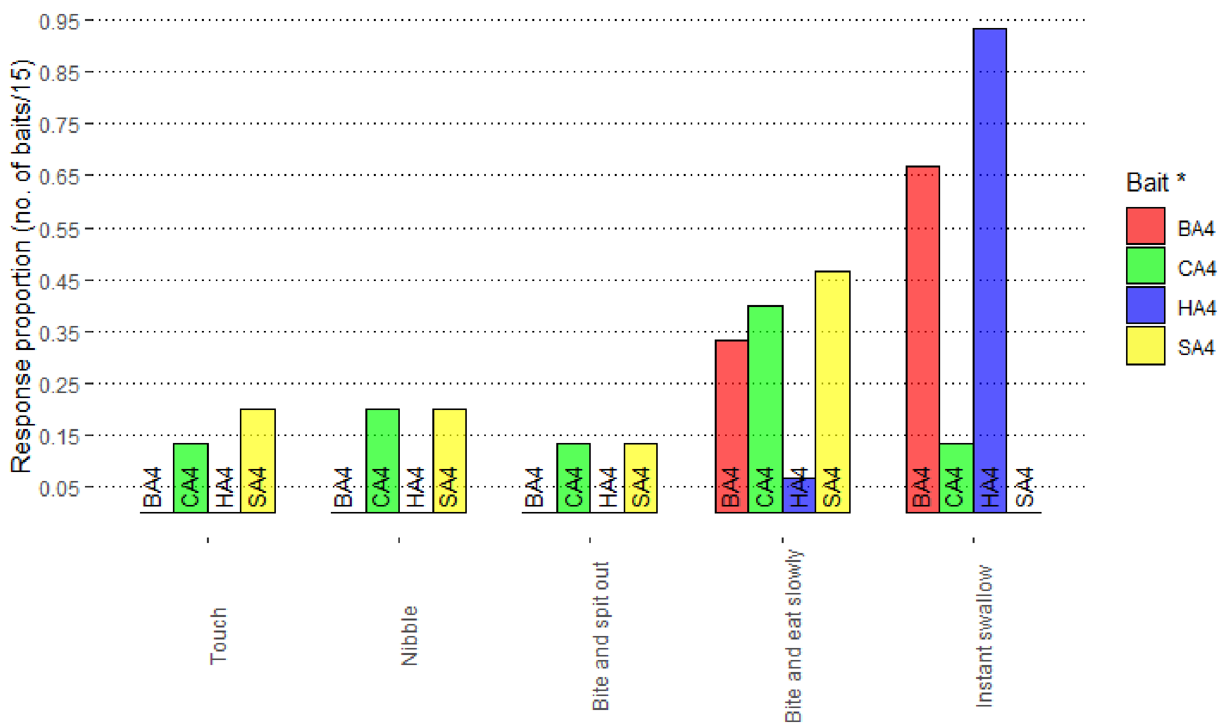
Due to unavailability of texture analyzer in the field, selection of baits with appropriate binder content was performed by simple observation and touching of the baits. The selection of baits for cod behavioral analysis was based on intact and coherent baits achieved with the preferably low content of binder to get constant and high leaching of attractants. It should able to be easily handled in the processing line without disintegration. Baits made with 2% alginate (HA2 and CA2) was weak during and after stuffing. The shape got collapsed at the cutting point. Hence it was cut carefully with a knife. The cylindrical shape was also readily disintegrable and break on slight touch. The amount of binder could not properly hold the minced fish making it sticky during shifting of the baits. It was more problematic in capelin baits (CA2). Increasing alginate powder by 2 % more (HA4 and CA4) gave highly intact bait and did not lose its shape after stuffing (clear-cutting) and during shifting of bait into the box and freezer. Increasing binder content strengthen the baits (HA6 and CA6), but one should compromise with leaching of attractants. In addition, blending was difficult and required high pressure during stuffing. Hence, only blending formulation with 4% alginate powder was used for making bait from other species as well and subjected all of them to cod behavioral analysis.

In TG-based baits, HTG0.5 and CTG0.5 (0.5% TG) were readily collapsible when scooped out from the mold after drying. CTG0.5 bait was even not able to form the hemispherical shape of the mold. While increasing the amount of TG by 1% (HTG1.5 and CTG1.5) and 2% (HTG2.5 and CTG2.5) did form the properly shaped bait with a hard crust, they were not suited well on rough handling owing to its softer core. Activa EB, Ajinomoto (microbial TG) claims to have strong restructured fish or meat with 1 to 1.5 % of its product when blended properly with pieces of raw materials followed by either vacuum packing or pressurized in the mold to get rid of air and let it set for 18-24 hours (Activa, n.d.). Lee et al. (1997) observed an increase in gel strength of cooked Alaska pollock surimi, the paste of which had been vacuum treated and packed before incubation, with the increase in transglutaminase content from 0 to 0.4 %. Mincing of fish in the present experiment might have incorporated the air and absence of such vacuum or pressure system during molding could have negatively affected the binding efficiency of the enzyme even in such high content of TG (Appendix 10.12). In contrary, fish balls made by rolling between the hands from a blend of minced tilapia and 1% Activa RM (transglutaminase) showed satisfactory binding properties (Huang & Clarke, 2017). Time and labor desired production with the loss of freshness in TG- based baits made a selection of alginate-based baits preferred over the former. However, it was subjected for behavioral analysis (HTG2.5 and CTG2.5) to know whether TG-based baits would be preferred by cod over alginate-based baits regardless of freshness.

6.2 Bait Group 2

6.2.1 Behavioral analysis

Behavioral analysis of cod towards different species of bait showed a varied degree of response. Figure 21 illustrates the difference in response scores of particular behaviors observed between four species of the bait (HA4, CA4, BA4 and SA4) for all of the five behavioral patterns namely ‘touch’, ‘nibble’, bite and spit out’, ‘bite and eat slowly’ and ‘instant swallow’. It is observed from the figure that the herring (HA4) and sprat (BA4) bait scored high in response frequency on the last phase of behavior profile of cod (i.e., instant swallow) compared to capelin (CA4) and saithe (SA4) bait. It means that herring and sprat baits were eaten instantly without any initial observations by cod, while about half of the capelin and saithe baits were inspected carefully as shown by touching, nibbling and spitting out behaviors and remaining were eaten slowly with considerable chewing as seen in video analysis. It shows higher preference of cod towards herring and sprat among four species in the experimental tanks which can have potential to be developed as alternative bait in pot fishing of Atlantic cod. A slight difference in cod behavior was also observed between herring and sprat baits where few sprat baits in the tanks were eaten slowly by the fish while most of the herring baits were engulfed immediately after feeding. From the observations, the order of species preference by cod can be arranged as HA4 > BA4 > > CA4 > SA4.



* Figure shows baits made from four different species of fish - sprat (B), capelin (C), herring (H) and saithe waste (S) with alginate (A) binder at the inclusion level of 4% indicated by their respective number alongside.

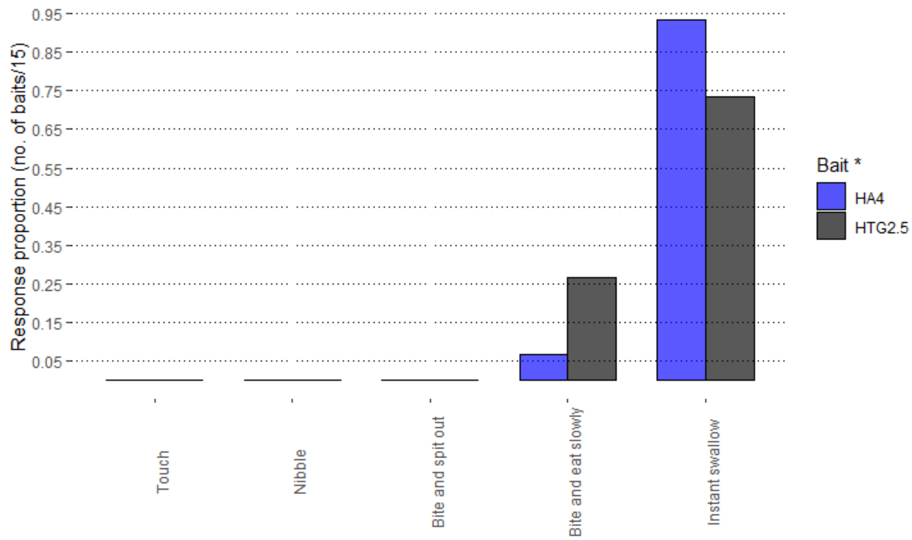
Figure 21. The proportion of four different species of baits responded by cod at a specific activity of the five behavioral patterns.

The highest preference of herring in present experiment was not consistent with the result obtained by Furevik and Løkkeborg (1994) who observed lower catching efficiency of cod from herring baits placed in pot traps in the north-western coast of Norway. Nevertheless, it was compared with highly preferred squid baits which have high market value and not relevant to our objectives. Ljungberg (2007), however, observed high catchment of cod baited with herring cuts in the Baltic Sea compared to squid bait and non-baited pots. Regardless of the difference in the geographical distribution of cod, the present experimental result could be related with the observations of stomach analysis of large cod conducted by Daan (1973) in the North Sea, where clupeids (Sprat and Herring) form the dominant food of the cod diet. Also, spawning NEA cod migrated along Norwegian coast feed largely on herring (Michalsen et al., 2008). Stomach analysis of cod in the Baltic Sea was observed about one-third of its diet with clupeids containing a higher proportion of European sprats and herring in medium and large size cod respectively (Pachur and Horbowy, 2013).

Icelandic cod and the NEA cod in the Barents Sea enormously feed on capelin (Dalpadado & Bogstad, 2004; Pálsson, 1983), in contrast to the behavior of local cod observed in the experimental tank where capelin bait did not show instant feeding response by the cod. This might refer to live prey feeding habit of cod (Tryggvadóttir et al., 2002) making them skeptical in recognizing their preferable prey in baited form or it might be due to geographical differences of cod. Siikavuopio et al. (2017) also observed capelin protein hydrolysates as an ineffective attractant producing the lowest frequency of interactions by cod in an experimental tank.

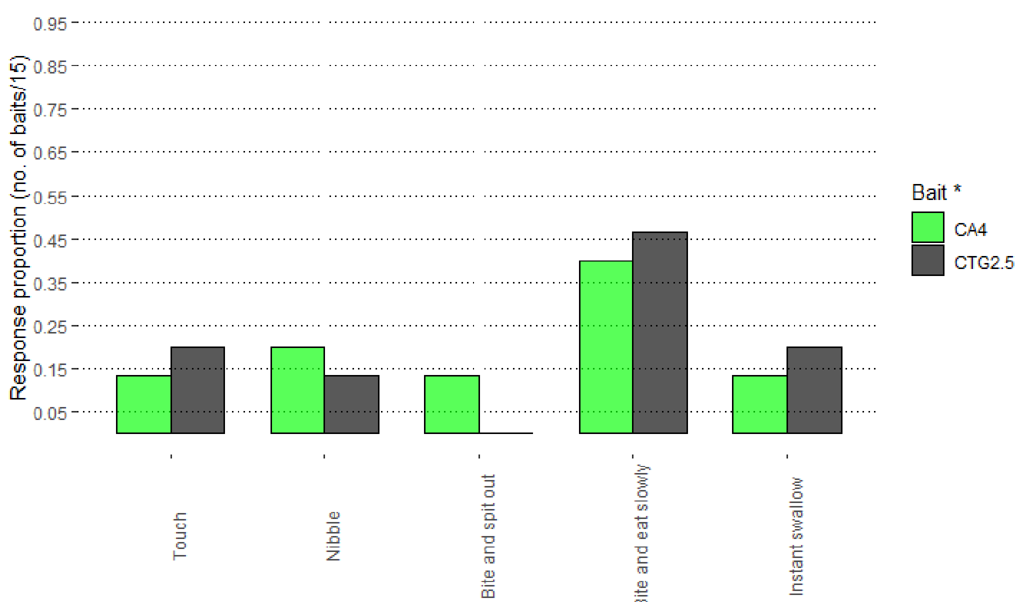
Saithe share the same family of cod i.e., Gadidae and are mostly found in North Atlantic. It has been reported a small proportion of gadoids in the diet of large cod but is mostly haddock and young cod (Daan, 1973). Bait made from saithe trimmings also showed the least response by cod in the present experiment.

Behavioral analysis of cod was also compared between the same bait using a different binder in minced herring and capelin. There wasn't observed notable difference in behavior of cod between alginate and TG-based baits of both herring and capelin. In herring bait, almost all the alginate-based baits were shown instance swallow response by the cod while it was slightly less (about 75% of total baits fed) in TG-based baits, the remaining baits being slowly eaten (Figure 22). This might be either of loss in the freshness of TG-based bait during drying or due to the difference in shape and texture from that of alginate-based baits or just because of chance variation. Both alginate and TG-based capelin baits showed a very small number of baits (less than 20%) responded instantly by cod (Figure 23).



* Figure shows baits made from herring (H) with alginate (A) and transglutaminase (TG) binder at the inclusion level of 4% and 2.5% indicated by their respective number alongside.

Figure 22. Proportion of two different types of herring baits responded by cod at specific activity of the five behavioral patterns.



* Figure shows baits made from capelin (C) with alginate (A) and transglutaminase (TG) binder at the inclusion level of 4% and 2.5% indicated by their respective number alongside.

Figure 23. Proportion of two different types of capelin baits responded by cod at a specific activity of the five behavioral patterns.

The response scores of the baits in 'Bait Group 2' were subjected to principal component analysis, and the data were graphically visualized in the form of biplot as shown in Figure 24. The PC1 in PCA biplot explains 82.4 % of the variance and PC2 explains 17 % of the variance in the data set. It portrays the main trend of the different baits towards the specific behavioral responses of cod and also shows the similarities and dissimilarities among these baits based on the responses. Baits described as highly preferable by cod lied on the same side (left) of the plot as separated by PC1 while the baits that were mostly not eaten (less degree of preference) as described by touch, nibble and spitting out behaviors lied on the other side. Baits that were shown a higher degree of instant swallow response lied on the bottom of the plot as separated by PC2 while those with a slow eating response on the upper side of the plot. The length of the straight line with the arrowheads explains the magnitude of the baits towards specific responses of cod. From the biplot, HA4 showed the highest magnitude towards the bait being instantly swallowed followed by HTG2.5 and BA4 to a lesser degree. CTG2.5, CA4, and SA4 were grouped together at the upper part of the biplot showing a slow eating response. However, the small length towards it and the tilt of the arrow on the right side of the plot also indicates the degree of uneaten response by cod.

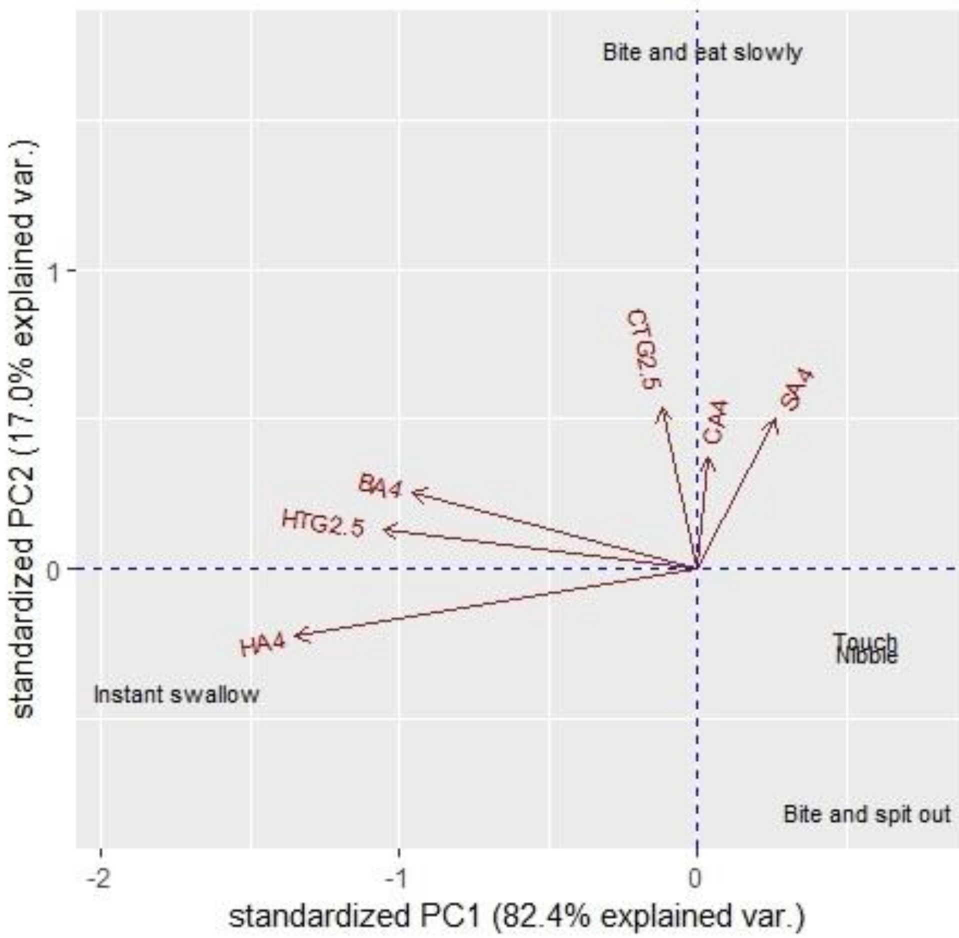


Figure 24. PCA biplot of behavioral response score obtained in various baits of 'Bait Group 2'

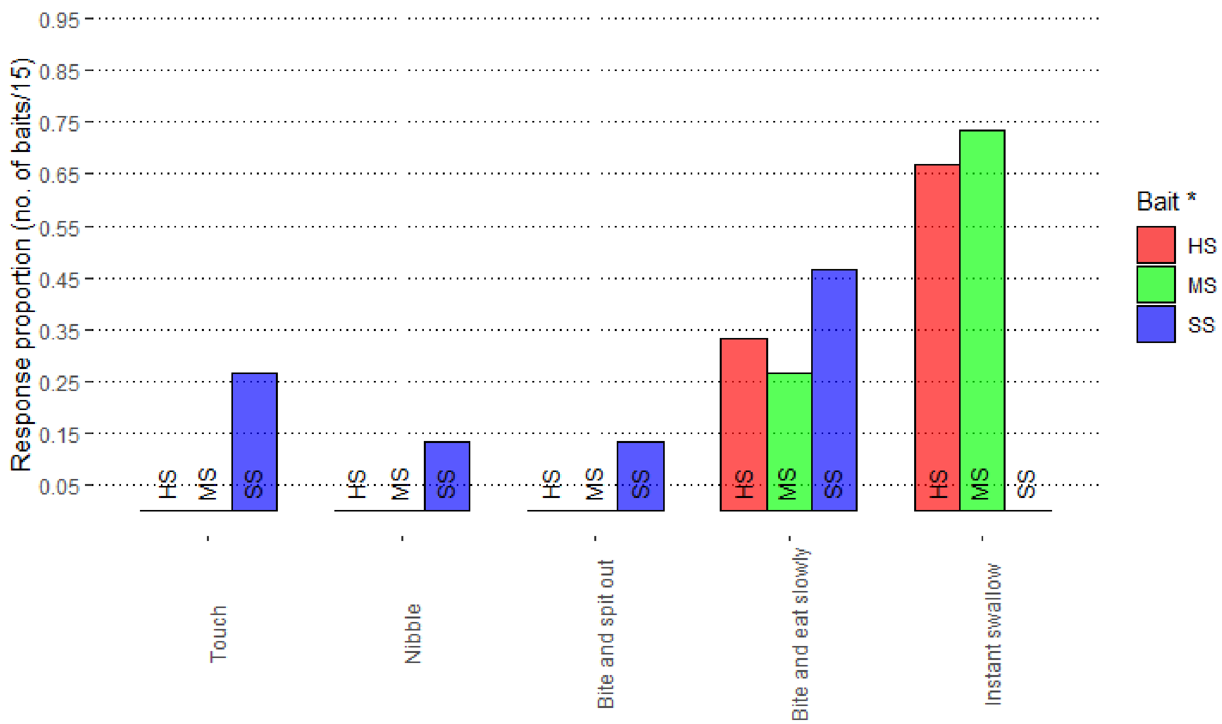
6.3 Bait group 3

Cooking of saithe fish skin with water (30g/100g of total weight) at 60 ºC for 10 minutes showed good gelling properties after setting it in chill condition for a night. Collagen is the structural unit which is most abundant in the skin. The water-insoluble fibrillar collagen when heated denatures to form a water-soluble hydrophilic colloidal protein called gelatin forming gel when cooled (Manjula et al., 2015; Sai-Ut et al., 2012).

Following the same procedure to obtain the manually deskinned herring (HS) and mackerel skin (MS) bait did not result in similar gelling ability as saithe skin bait (SS). Instead, they readily collapsed on soft handling. A layer of fat could be easily seen over both the baits, as shown in Figure 11, which might be the reason for its poor gelling ability. Herring and mackerel are fatty fish containing more than 20% of lipid (on a wet basis) compared to lean saithe fish (less than 1%) (Smith & Hardy, 2001; Tryggvadóttir et al., 2002).

6.3.1 Behavioral analysis

Regardless of the weak bait formed (HS and MS), they were tested for behavioral analysis of cod which showed better performance in provoking an instant response in cod over saithe skin baits (Figure 25). More than 65% of the MS and HS baits were instantly swallowed, and the remaining were taken in and out of the mouth by some fishes before they were completely swallowed. On the other hand, almost all SS baits showed primary responses including touching, nibbling and spitting out without eating in two tanks while the cod in the third tank slowly ate all five baits with a high degree of initial inspections. SS baits were significantly less in triggering instant swallow response by cod than MS and HS baits. The preference of baits can be placed in order of MS > HS >>> SS, triple arrows indicating very less preference of saithe skin by cod. Preference of herring skin over saithe skin could be related to its preference for whole minced herring baits as explained before. Mackerel cuts as bait for cod have performed well in few studies done, and this might be the reason for highest preference of the bait made from its skin. Johnstone and Hawkins (1981) observed mackerel cuts as potent bait in longline fishing of cod conducted in Scottish sea compared to least effective salted herring baits. Mackerel and herring have also been found as one of the dominant diets of cod in the northeast US continental shelf (Link & Garrison, 2002).



***** Figure shows baits made from three different species of fish skin – herring skin (HS), mackerel skin (MS) and saithe skin (SS).

Figure 25. Proportion of three different skin baits responded by cod at specific activity of the five behavioral patterns.

Despite showing good behavioral performance by cod, inability to form a strong gel from mackerel and herring skin have failed them to fulfill the requirement as bait for longline fishing, while this was the other way around for saithe skin which formed a strong gel but lacked in scoring good sensory results for cod. This could be improvised either by strengthening weak gel by addition of gelling agents like gelatin which might have a negative effect on leaching of natural attractants or by incorporating synthetic attractants on a strong substrate of natural origin. The later was followed in present experimental work.

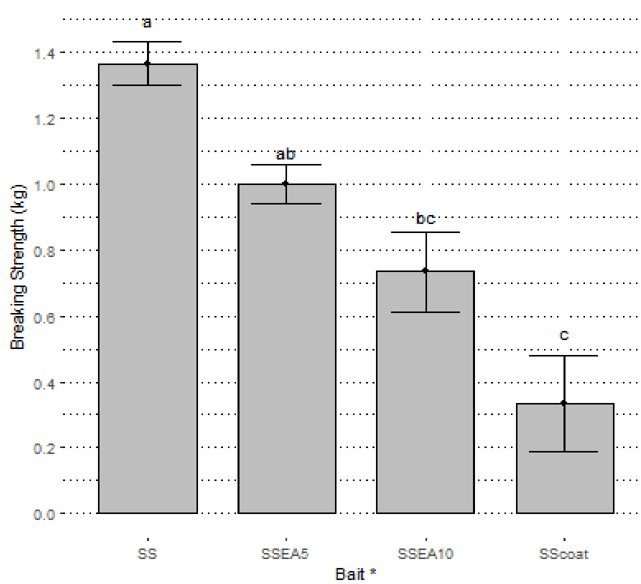
6.4 Bait group 4

Addition of Ecobait attractant (EA) at the level of 5% (SSEA5) and 10% (SSEA10) in saithe skin solution and use of fine solution of the skin as coating layer over instantly responded herring (HA4) bait (SScoat) improved the behavioral pattern of the cod but at the same time, declined its hooking strength relative to pure skin bait (SS).

6.4.1 Breaking strength

The tensile force measured in 1cm skin layer (SS, SSEA5, and SSEA10) and whole SScoat showed the decrease in value at breaking point. The mean breaking strength of SS layer was 1.37 ± 0.12 kg which decreased linearly with increase in attractant addition (SSEA5 and SSEA10) and further to the lowest mean value of 0.33 ± 0.25 kg in SScoat as shown in Figure 26. The variance obtained in SScoat was perhaps large owing to the differential thickness of the coated skin layer and the device hook that needs to be passed through minced herring core in between the layers during analysis. Statistical analysis showed SS bait significantly different with SSEA10 and SScoat but not with SSEA5. SSEA5 was not significantly different with SSEA10 but significantly different with SScoat. There was no significant difference between SSEA10 and SScoat.

The decrease in strength of skin bait with increasing level of attractants can be attributed to the negative effect of liquid protein hydrolysates of fish product (EA) used as attractants for cod. Fine grinding of skin solution used as coating layer and structural integrity of SScoat might result in lowest strength of the coated bait.



***** Figure shows three saithe skin baits (SS) made with Ecobait attractant (EA) at 0, 5 and 10 % level of inclusion indicated by their respective number and one saithe skin coated herring bait (SScoat). Different superscript represents a significant difference between the baits at $p < 0.05$.

Figure 26. Breaking strength (kg) of four types of skin baits

6.4.2 Hooking stability

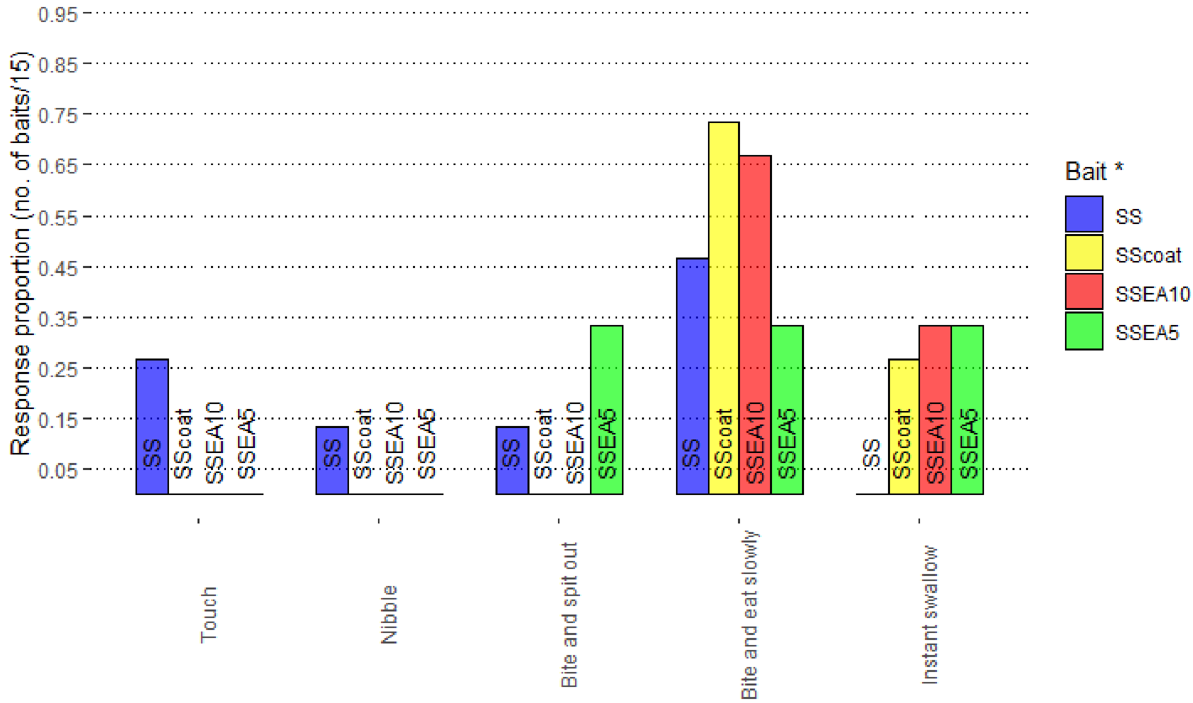
All the baits (3 baits per trial) of Bait group 4 were stable in the hooks for 12 hours against the water current maintained in the tank through the pump. Appendix 10.10 shows three baits (SScoat) of the lowest strength but is still stable in water after 12 hours of standing.

Although the baits were stable in fast flowing water in the experimental tank, it cannot be concluded unless realistic testing in the field is done where several factors including repetitive attacks from different fish and scavengers account for poor hooking strength and loss of baits. It should also withstand handling in baiting machine during the shooting of hooks over the baits. Squid bait has been considered ideal bait for longline fishing gears due to its strength and minimal bait loss (He, 1996). The knowledge obtained from the experiment is nevertheless significant as a step ahead towards the development of an ideal bait from the skin which requires further improvisations.

6.4.3 Behavioral analysis

The aim of improving sensory characteristics of the saithe skin bait to attract cod has been productive to some extent. Compared to pure skin baits (SS), which didn't provoke efficient eating motivation in cod, the addition of attractants to 5 and 10 % gradually increased their eating tendency (Figure 27). Cent percent of SSEA10 and SScoat baits were eaten in three tanks while all of the five SSEA5 baits fed in tank 2 were spit out which lowered its overall response value. SSEA10 and SScoat showed a similar response in the last two phases of behavioral pattern. Both baits showed primary inspections by cod before they were eaten in two tanks (1 and 2) while cod in the third tank responded instantly for all the three baits (SSEA5, SSEA10, and SScoat) (see Appendix 10.6). The instant response in tank 3 indicates the change in the behavior of the wild cod in the experimental tank kept for a long time as 'Bait group 4' was subjected to behavioral analysis on the last period of the experiment. This might have influenced in obtaining the actual behavioral pattern of cod as seen in real circumstances and calls for replacing with new ones if possible or timely reshuffling of fishes within the tanks.

This result demonstrates that improvisation of taste parameter in saithe skin baits while maintaining the strength might help in developing fish skin as potential bait for longline fishing of cod.



***** Figure shows three saithe skin baits (SS) made with Ecobait attractants (EA) at 0, 5 and 10 % level of inclusion indicated by their respective number and one saithe skin coated herring bait (SScoat).

Figure 27. Proportion of four different skin baits responded by cod at specific activity of the five behavioral patterns.

The response scores of the skin-based baits of 'Bait Group 3' and 'Bait Group 4' were together subjected to principal component analysis, and the data were graphically visualized in the form of biplot as shown in Figure 28. The PC1 in PCA biplot explains 70.9 % of the variance and PC2 explains 24.7 % of the variance in the data set. Baits described as highly preferable by cod lied on the right side of the plot as separated by PC1 while the baits that were mostly not eaten (less degree of preference) as described by touch, nibble and spitting out behaviors lied on the left side. Baits that showed a higher degree of instant swallow response lied on the bottom of the plot as separated by PC2 while those with a slow eating response on the upper side of the plot. From the biplot, MS and HS showed the highest magnitude towards the bait being instantly swallowed and SScoat and SSEA10 towards the baits being slowly eaten. SS bait was the least preferred bait shown by slightly tilted line towards 'bite and eat slowly' response with less magnitude of arrow-line indicating weak response on that quarter and some inherent response of the left side (touch, nibble, bite & spit out).

SSEA5 lied on the right side of the plot with the least magnitude indicating better eating response than SS but still having properties of left sides of the plot.

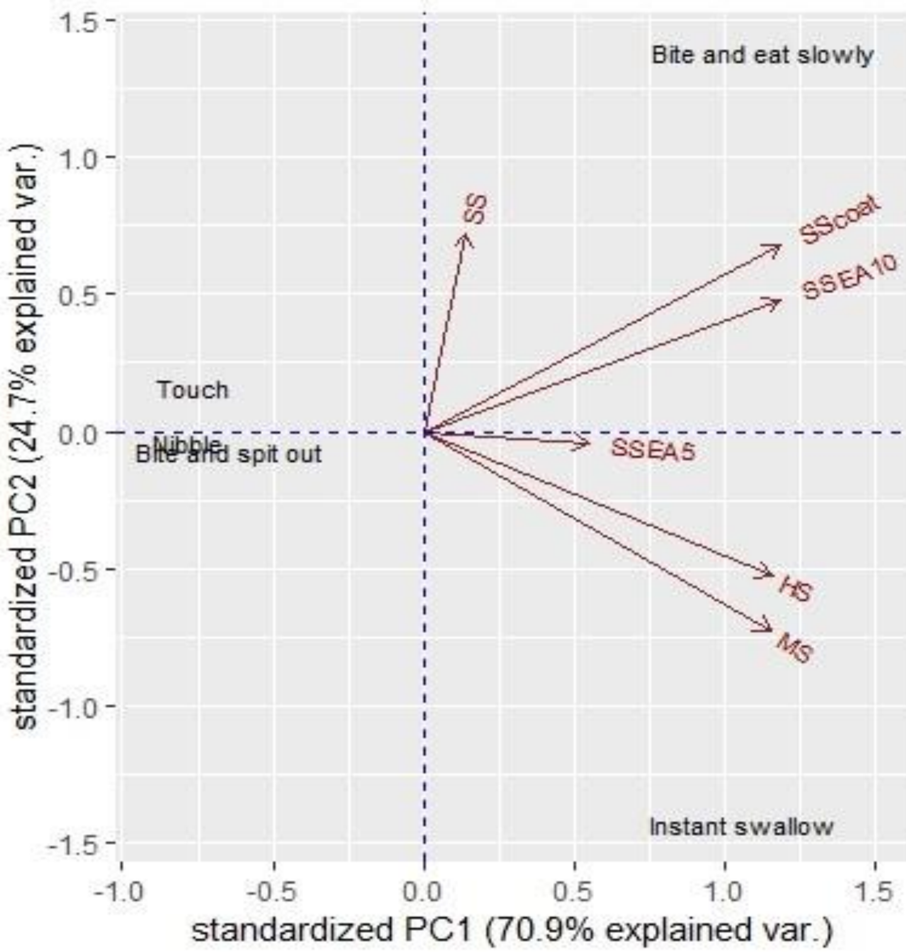


Figure 28. PCA biplot of behavioral response score obtained in various skin baits.

7. Conclusion

This study has shown that the fresh baits from minced fish and trimmings can be developed for cod pots at the minimum level of 4% alginate binder with satisfactory handling properties and water stability obtained by simple stuffing technique. Microbial transglutaminase as the binding agent requires more time and labor demanding improved processing steps to form the good bait. Cod exhibit various degree of preference for the baits of different species of fish when fed in an experimental tank. Herring and sprat baits are highly preferred by cod compared to capelin and saithe baits.

Study of skin baits made from the three different species of fish showed saithe skin as an ideal bait regarding strength that could possess potential to sustain competently in baiting machine and longline hooks. Unfortunately, it lacks key characteristics to attract cod to be potentially hooked. Improvisation of taste factor in saithe skin-based baits can be achieved by admixing synthetic attractants or using skin as coating layer over the baits of highly attractive fish. However, it still requires further research to attain better strength and taste of improvised skin baits for longline fishing of cod.

Being the initial phase of the project, the present study lacks strong directions to wholeheartedly support the result of the experiment for implementing it commercially. Nevertheless, it provides a great deal of knowledge and a solid foundation for correcting and improvising processing and analytical technique in the next phase of the project.

8. Future outlook

This study has shown that alginate can be used as the binder in achieving suitable bait from the waste, low valued and surplus fish by mincing and stuffing technique for the pot fishing of cod. The study, however, requires better standardization of processing line and additional analytical methods on the field such as analysis of textural properties and leaching rate of attractants, before it is approved for industrial processing of baits. This applies same for skin baits and their modified form for longlining. Further improvement in strength of preferred skin baits like mackerel and herring skin could be done by extraction of fat and innovate other process variations. Skin-coated baits can have great potential to exploit the symbiotic effect of more than one species of attractive fish in a single bait for longline fishing of cod if further studies could be done utilizing advanced coating and layering technology existing today.

Analytical system for the study of behavioral responses of cod in the experimental tanks needs to be improvised to understand their response against the baits correctly. A small volume of tanks and presence of single camera prevent us from analyzing series of complete behaviors acted upon a single bait due to the view blocked by the fishes while swimming in front of the camera and their frequent movement outside of the filming zone with baits around their mouth. This compelled us to study the overall behavior pattern acted on single bait and the response value scored as the number of baits rather than recording the score as the frequency of different behavioral pattern occurred on the single bait. In addition to controlled light and temperature management, an automated system for feeding should be developed that can feed the baits preventing the possible human interference near the tanks. The response behavior might be more reliable if the cod are replaced or interchanged timely which can help reduce the rate of adaptation of wild cod in the experimental environment.

Systematic studies with other species of fishes, crustaceans, mollusks, and planktons could be done in the laboratory to study more about the preferred prey species of cod. The highly preferred prey species can then be selected and analyzed chemically to recognize their potent attractants. These species can be used directly in the baited form or subjected to controlled hydrolysis to obtained protein hydrolysates which can be used as attractants over other substrates suitable for baited gear fishing. Thermal-pressure treatment like an extrusion of attractive prey species or their hydrolyzed products with cereals can also be an innovative method in bait production. All the experimental result obtained from the laboratory studies should be verified systematically by the results of real field testing and commercial fishing trials.

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10. Appendix

10.1 Moisture content (%) of herring and capelin baits of 'Bait Group 1'

Herring Baits	Mean ± SD	Capelin Baits	Mean ± SD
HA2	67.62 ± 0.27	CA2	73.14 ± 0.28
HA4	65.52 ± 0.11	CA4	72.12 ± 0.08
HA6	63.32 ± 0.16	CA6	70.59 ± 0.28
HTG0.5	59.70 ± 1.07	CTG0.5	67.30 ± 0.56
HTG1.5	59.80 ± 1.26	CTG1.5	67.29 ± 1.41
HTG2.5	59.19 ± 0.74	CTG2.5	66.22 ± 0.49

10.2 Water activity of herring and capelin baits of 'Bait Group 1'

Herring Baits	Mean ± SD	Capelin Baits	Mean ± SD
HA2	0.966 ± 0.002	CA2	0.963 ± 0.001
HA4	0.965 ± 0.001	CA4	0.961 ± 0.001
HA6	0.964 ± 0.001	CA6	0.959 ± 0.001
HTG0.5	0.962 ± 0.001	CTG0.5	0.963 ± 0.001
HTG1.5	0.962 ± 0.001	CTG1.5	0.962 ± 0.000
HTG2.5	0.962 ± 0.001	CTG2.5	0.959 ± 0.001

10.3 Water stability (%) of herring and capelin baits of 'Bait Group 1'

Herring Baits	Mean ± SD	Capelin Baits	Mean ± SD
HA2	77.55 ± 3.04	CA2	46.32 ± 5.89
HA4	84.64 ± 4.02	CA4	60.82 ± 4.76
HA6	86.88 ± 4.70	CA6	64.35 ± 2.47
HTG0.5	81.98 ± 5.30	CTG0.5	77.47 ± 5.24
HTG1.5	86.06 ± 5.20	CTG1.5	83.60 ± 3.10
HTG2.5	89.06 ± 1.26	CTG2.5	87.23 ± 2.52

10.4 Behavioral response score (as number of baits) of 'Bait Group 2'

Behavior	**Baits**																	
	HA4			**CA4**			**BA4**			**SA4**			**HTG2.5**			**CTG2.5**		
	Tank			Tank			Tank			Tank			Tank			Tank		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Touch	0	0	0	2	0	0	0	0	0	1	2	0	0	0	0	3	0	0
Nibble	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0	2	0	0
Bite and spit out	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0
Bite and eat slowly	1	0	0	0	3	3	1	2	2	2	0	5	2	2	0	0	4	3
Instant swallow	4	5	5	0	0	2	4	3	3	0	0	0	3	3	5	0	1	2

10.5 Breaking strength (kg) of skin baits of 'Bait Group 4'

Baits	SS	SSEA5	SSEA10	SScoat
Mean ± SD	1.37 ± 0.12	1.00 ± 0.10	0.73 ± 0.21	0.33 ± 0.25

10.6 Behavioral response score (as number of baits) of 'Bait Group 3' and 'Bait Group 4'

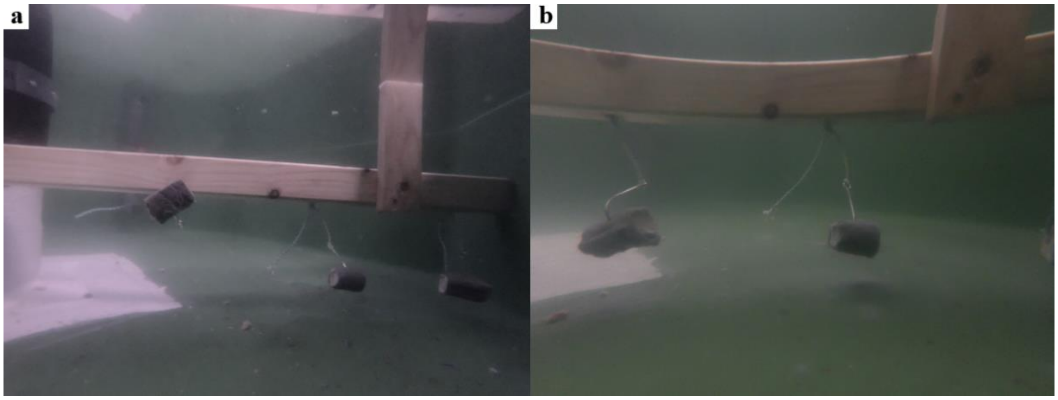
Behavior	**Baits**																	
	HS			**MS**			**SS**			**SSEA5**			**SSEA10**			**SScoat**		
	Tank			Tank			Tank			Tank			Tank			Tank		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Touch	0	0	0	2	0	0	2	2	0	0	0	0	0	0	0	0	0	0
Nibble	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Bite and spit out	0	0	0	0	2	0	2	0	0	0	5	0	0	0	0	0	0	0
Bite and eat slowly	1	2	2	1	1	2	1	1	5	5	0	0	5	5	0	5	5	1
Instant swallow	4	3	3	4	4	3	0	0	0	0	0	5	0	0	5	0	0	4

10.7 Rotronic hygrolab – water activity measuring device

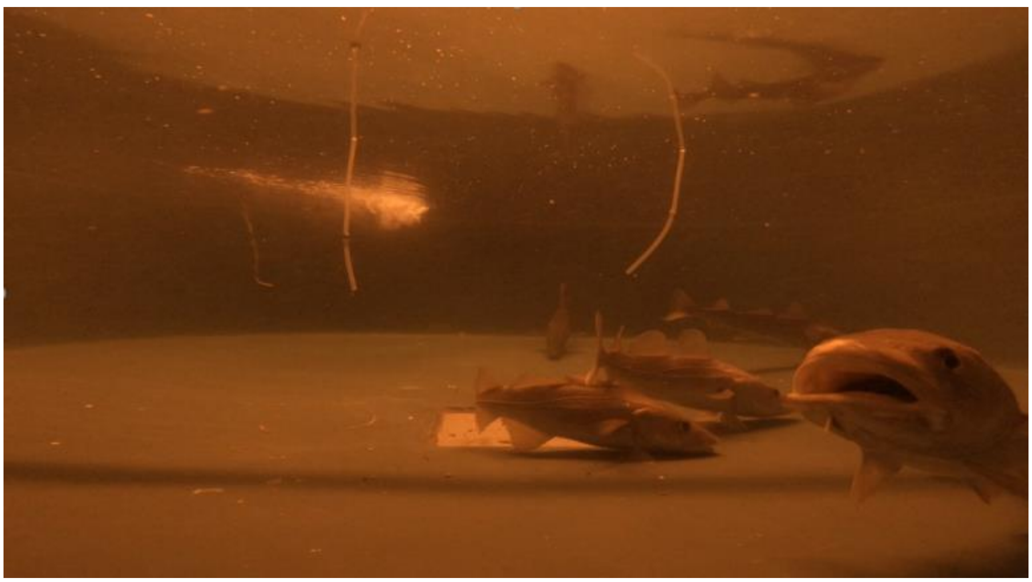
10.8 Perforated cylindrical box for water stability analysis of baits in sea.

10.9 Julabo SW22 – water bath shaker for analysis of water stability of baits in laboratory

10.10 Experimental tank for measuring hooking stability of baits – (a) SScoat baits at 0 hour (b) SScoat baits at 12 hours.



10.11. Experimental tank for behavioral analysis of cod against the baits. (Picture taken from GoPro camera fitted in the tank)



10.12 Cross-sectional view of TG-based herring bait (HTG2.5)

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