Fisheries Research 227 (2020) 105535

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Feeding response of Atlantic cod (*Gadus morhua*) to attractants made from by-products from the fishing industry

Anne Christine Utne-Palm[a,*], Andre S. Bogevik[b], Odd-Børre Humborstad[a], Tone Aspevik[b], Michael Pennington[a], Svein Løkkeborg[a]

[a] Institute of Marine Research, PO Box 1870, N-5817, Bergen, Norway
[b] Nofima - Norwegian Institute of Food, Fisheries and Aquaculture Research, Kjerreidviken 16, N-5141, Fyllingsdalen, Norway

ARTICLE INFO

Handled by George A. Rose

Keywords:
Attractant
Alternative bait
Atlantic cod
Longline
Feeding behavior

ABSTRACT

The aim of this behavioural study was to identify potential feeding attractants to be incorporated in an alternative longline bait for Atlantic cod. The attractants should be based on low-cost surplus resources that are not used for human consumption. The food search and feeding responses of wild caught cod to eight attractants made from products from the fishing and aquaculture industry were compared to traditional squid bait in a laboratory study. All attractants tested triggered feeding responses in cod, indicating that there are several by-products from the fishing industry that have potential as an attractant for an artificial longline bait. The three most effective attractants were herring processing by-products, sand eel hydrolysate and hydrolysate by-products from the shrimp industry, which all elicited stronger food search and feeding responses than squid. Our results indicate that both free amino acids and other unidentified compounds are important in eliciting feeding responses in cod. Thus, attempts to identify efficient feeding attractants to be incorporated in alternative baits should be based on using complete aqueous extracts, rather than isolating a mixture of potent components.

1. Introduction

Annual landings of Atlantic cod (*Gadus morhua*) by the Norwegian longline fleet range from 64 000 to 74 000 tons (2014–2017), which is 16 % of the total cod catches (data from the Directorate of Fisheries). The longline vessels use more than 10 000 tons of bait a year (Løkkeborg, 2013). Baits commonly used by the Norwegian longliners comprise squid (*Illex* spp.), mackerel (*Scomber scombrus*), herring (*Clupea harengus*) and Pacific saury (*Cololabis saira*). These resources are also used for human consumption, and there is a growing demand worldwide for food (FAO et al., 2015), a better and more sustainable use of marine resources (Frid and Paramor, 2012; FAO, 2018). In addition to this ethical dilemma, there is an economic problem because prices for traditional baits have greatly increased in the course of the last two decades (Løkkeborg, 2013; www.fiskernes-agnforsyning.no). This price increase is driven by the growing demand for human food resources. Thus, it is important to find alternative bait products that are not used for human consumption.

An alternative longline bait attractant should be effective (initiating food search and feeding behavior), species-selective and based on low-cost by-products. Such products need to be available in large quantities to meet the high demand for bait. Based on criteria such as quantity, availability, price and chemical composition, potential resources that are not used for human consumption includes species caught in the Norwegian pelagic fisheries and processed into fish meal and oil: Norway pout (*Trisopterus esmarkii*), blue whiting (*Micromesistius poutassou*), capelin (*Mallotus villosus*) and sand eel (*Ammodytes marinus*). In addition, the fishing and aquaculture industry produces large quantities of fish by-products. While Norwegian processing plants for landed pelagic species (herring and mackerel) and farmed salmonids utilize 90–100 % of their by-products for different purposes (e.g. animal feed), only 40–60 % of white fish by-products are utilized (Winther et al., 2013; www.barentswatch.no). Manufactured longline baits based on these alternative sources should have a potential for catching cod since cod is a predatory fish with a broad diet (Klemetsen, 1982, and references within).

The marine by-products could be included in the manufactured baits “as is” or as fish meal or fish oil if stored properly after catch. Low molecular weight (LMW) compounds from hydrolyzed by-products are known to stimulate appetite (Carr and Derby, 1986) and could be preferably produced as attractants in baits. This could either be produced by the use of exogenous enzymes (hydrolysate) or endogenous

---

* Corresponding author.
E-mail address: annecu@hi.no (A.C. Utne-Palm).

https://doi.org/10.1016/j.fishres.2020.105535
Received 4 September 2019; Received in revised form 6 February 2020; Accepted 11 February 2020

enzyme in combination with formic acid (silage). The enzymes will cleave the proteins into smaller and more water-soluble peptides and free amino acids. The production of fish silage can easily be established on vessels and appears more cost-efficient due to its utilization of activity of tissue degrading enzymes that are naturally present in the fish, mainly the viscera (Raa and Gildberg, 1982). The silage process usually requires long hydrolysis time and the products are not suited for human consumption. On the other hand, the production of protein hydrolysates by the use of exogenous enzymes results in higher and more predictable quality of the end-product, and is the preferred way of utilizing by-products for human consumption. However, this method is more costly, mainly due to need for commercial enzymes.

Chemoreception is of paramount importance for the detection and location of small, stationary food items such as baits (Løkkeborg, 1998; Løkkeborg and Fernö, 1999). Thus, feeding attractants comprise an important component of an efficient longline bait. Studies have demonstrated that feeding behavior in fish is stimulated by LMW molecules with high aqueous solubility and amino acids appear to be the most stimulating group of compounds (Carr and Derby, 1986; Hara, 1992, 2011; Kasumyan and Døving, 2003). Several studies have also demonstrated that there is great potential for using baits or extract mixtures to attract specific target species (reviewed by Løkkeborg et al., 2014). Efforts to develop alternative longline baits to replace traditional baits have been made in several countries (e.g. Bjordal and Løkkeborg, 1996; Januma et al., 2003; Pol et al., 2008; Henriksen, 2009), but to date these efforts have generally not been successful because the catching efficiency of these baits types has proved to be inferior compared to traditional bait types (Løkkeborg et al., 2014). Thus, only natural and traditional baits are currently used worldwide by commercial longline vessels

Food search behavior is commonly species-specific, and thus there is a great potential for improved species selectivity in longline fishing by incorporating species-specific attractants in manufactured baits (Løkkeborg et al., 2014). The main objective of this study was to test the behavioral response of cod to attractants made from marine sources not used for human consumption. Further, to test if the processing method may enhance the stimulatory capacity of a bait, we tested one bait (sand eel) as fresh, hydrolysate and silage. These attractants were compared with an attractant based on squid which is the most common bait used by Norwegian longline vessels targeting cod (Bjordal and Løkkeborg, 1996; Løkkeborg, 2013). As a first step in an effort to develop an alternative bait, these trials were carried out in large tanks to identify the most promising attractant sources (better or as good as squid) for later incorporation in a bait matrix that can be tested under commercial conditions.

2. Material and methods

2.1. The experimental fish

The cod used in this study were caught by a Danish seiner in the Barents Sea, south-east of Svalbard (between 77°5´and 78.2´N, 26°4´ and 33.5´E) at depths of 110–230 m (temperature 3 °C). The fish were caught between 25th and 28th of September 2016 and transported to the Tromsø aquaculture research station following the procedure for fish handling in capture-based aquaculture (CBA). This procedure includes sorting out and slaughtering all fish with positive buoyancy, visible injuries or reduced vitality (Humborstad et al., 2016; Humborstad and Mangor-Jensen, 2013; Humborstad et al. 2009), before transferring the remaining fish to specialized live capture tanks with perforated double bottoms (Humborstad et al., 2016). At the research station the cod were transferred to an outdoors fiberglass holding tank (3 m diameter, water depth 1 m ~ 7 m^3 of water), which had a constant flow of seawater at ambient temperature 9 °C (± 0.6). The cod (N = 50) had an average length of 73 cm (± 5.2 SD). The tank was covered by a black fine meshed netting (10 mm half mesh) to keep birds out and still keep the light level natural. The fish were acclimatized for two weeks in the holding tank before the experiment began and were not fed during the experiment to ensure feeding motivation. According to Norwegian regulations (FOR-2004-12-22-1878, §95) wild-caught fish can be held for four weeks without feeding, and the tolerable fasting period for cod at good biological condition is 54 days (Ageeva et al., 2017). The maximum duration a single cod was held without feeding was 25 days (range 12–25 days). We observed no mortality or injury in held fish.

2.2. Choice of attractant sources, preparation and composition

Fish meal used by the aquaculture industry in Norway are to a large extent produced from pelagic fish not used for human consumption (e.g. sand eel) and by-products from pelagic species such as mackerel and herring. High quality by-products from the salmon industry and white fish fisheries, such as heads and backbones, are mainly processed into fish protein products for pets and humans. Low-quality fish, and fish by-products, not useable for human consumption are preserved in formic acid as silage and processed into fish protein concentrates (fat is separated out) that are cheap and readily available. In the present study, silage based on salmon (mainly viscera) and whitefish (cod and haddock; heads, backbone and viscera), manufactured by Scanbio AS (Trondheim, Norway) were tested. From the fish meal species, sand eel was chosen to be included in the study, which is a low-priced product not used for human consumption (Table 1). Moreover, yeast extract, hydrolysate based on by-products from the Northern shrimp (*Panadalus borealis*) industry, and herring processing by-products (after removing filets) were used. The same shrimp hydrolysate has previously been tested as a cod attractant and it was found to be superior to hydrolysates based on capelin (*Mallotus villosus*) and blue mussel (*Mytilus edulis*) (Siikavuopio et al., 2017). The shrimp hydrolysate was made by an enzymatic hydrolysis processes described by Kristinsson (2007). Yeast extract is commonly used as an attractant in leisure fishing, and it contains high levels of betaine and nucleotides, which is known to stimulate appetite in marine species (Carr and Derby, 1986). Yeast is easy to store, highly available and low priced. Further, herring by-products was chosen, as herring is commonly used in the Norwegian longline fishery targeting cod (Løkkeborg, 2013). Squid being the most commonly used longline bait in Norway, was used as a reference bait.

Table 1

Potential bait products. The listed baits are baits already in use (squid, mackerel and herring) or products that have a potential to be used, based on availability and price. Volume and prices are given for the period 2012-2017 in Norway.

Species	Weight (Tons)	Price (NOK/kg)	Products
Capelin	76 000–270 000	1.70–2.38	Not human food
Norway pout	3000–47 000	1.70–2.20	Not human food
Blue whiting	20000–399 000	1.45–2.34	Not human food
Sand eel	30 000–108 000	1.70–2.14	Not human food
Copepods	520[1]	variable	Not human food
Krill	93 000–179 000	0.43–0.51	Not human food
Salmon by-products[2]	30 % of total weight	0.50–1.50	Not human food
Shellfish by-products	119-213 000	6.47–4.40	Not human food
Squid[3]		25.25	Human food / bait
Mackerel[4]	230 000	2.75-3.50	Human food/ bait
Herring[4]	455 000	2.25	Human food/ bait
Herring by-products[4]	455 000	2.55	Not human food

[1] Norwegian test fishing by *Calanus AS*, price is depended on quality and volume.

[2] Prices given by the Norwegian salmon slaughterhouses.

[3] Prices given by “Fiskernes Agnforsyning”.

[4] Prices and weight are from “Norges Sildesalgslag”.

2.2.1. Production of products based on sand eel

Frozen sand eel (delivered at landing sites in southern Norway) was ground twice in a kitchen grinder (aperture 7 mm) before being prepared in three different ways to test if the processing method would enhance the stimulatory capacity of the product: 1) frozen directly at -20 °C, 2) prepared as a fish silage product and 3) hydrolyzed by exogenous enzymes. In the silage process, ground sand eel was mixed with formic acid (Helm FS +, Hjelle Kjemi, Norway) to pH > 4 and kept for two days at room temperature (22 °C) under continuous mixing. The final liquid silage was stored in plastic containers in a refrigerated room (3 − 5 °C) until use. In the hydrolysis process, ground sand eel was mixed with distilled water (1:1) and the temperature adjusted to 50 °C. The enzymes Protamex and Flavourzyme 1000 L (Novozymes, Bagsværd, Denmark) were added at 0.5 % w/w (dry matter basis) and the hydrolysis run for two hours. Subsequently, the hydrolysis slurry was heated to > 90 °C in a microwave oven and kept at this temperature for 10 min to inactivate the enzyme activity. The resulting hydrolysis slurry was cooled down, transferred to plastic containers and kept frozen (-20 °C) until use.

2.2.2. Preparation of attractants

The shrimp attractant was made by Polybait AS (Tromsø, Norway), and is a powder made through an enzymatic hydrolysis processes described by Kristinsson (2007). The frozen sand eel, frozen gutted squid and fresh herring head, bone and viscera (HBV), were all ground in a food processor (shredding disc opening 5 mm). Following, the attractant solutions were prepared by soaking 1 kg of the ground product in 10 l of filtered seawater for 12 h (see Supplementary Fig. 1). For the silages, hydrolysates and yeast extract, it was back calculated so that the same ratio between raw material (1 kg) and distilled water (10 l) was used (see Supplementary Table 1). After soaking (12 h), the attractant solutions were filtered through an 80 µm plankton filter, packed in 250 ml portions and frozen (−20 °C) until used in the fish experiment.

The attractant solution used in the fish experiment was analyzed for crude protein by the Kjeldahl method (ISO 5983-2, 2009), total fat (Folch et al., 1957) and dry matter (ISO 6496-2, 1999). The attractant solution had dry matter content of 4.3–6.2 %, composed of 0.6–2.1 % crude protein and 0.1–0.7 % total fat. Yeast extract showed the highest content of protein, with as much as 82 % being water-soluble. Similar, processed marine attractants (hydrolysates/silage) had a much higher content of water-soluble proteins compared to non-processed (herring, sand eel and squid). The pH in the solutions reflected the processing, with a lower pH in silage products compared to the other attractants (Table 2).

The water-soluble fraction of the attractant was analyzed for peptide size distribution (Wang-Andersen and Haugsgjerd, 2011) and composition of free amino acids (Bidlingmeyer et al., 1987), in addition to total amino acid composition (Liu et al., 1995). Proteins are being hydrolyzed during storage and processing to smaller and more water-soluble peptides, where size of peptides and levels of free amino acids mostly depend on enzymes present and processing conditions. All the attractants had high levels of low molecular peptides (< 0.2 kDa) consisting mainly of free amino acids (Fig. 1a and b). The yeast extract and processed fish (silage/hydrolysates) demonstrated the highest levels of small peptides (< 1 kDa), while only untreated sand eel, gutted squid and herring HBV contained significant levels of larger peptides > 1 kDa.

Table 2

Dry matter, crude protein, total fat and pH in solutions of attractants.

Samples	Dry matter (%)	Crude protein (%)	Total fat (%)	pH
Sand eel hydrolysate	4.8	1.1	0.74	6
Herring	4.7	1.0	0.47	6
Shrimp hydrolysate	4.4	0.6	0.09	8
Squid (gutted)	4.3	0.7	0.09	7
Salmon silage	4.3	0.8	0.26	4
Sand eel silage	5.2	1.2	0.74	4
Sand eel	4.5	0.8	0.48	6
Yeast extract	6.2	2.1	0.07	7
White fish silage	4.4	0.7	0.08	4

The total amino acid composition in the samples (Table 3) reflected the protein content (Table 2), with highest content in sand-eel silage and lowest content in shrimp hydrolysates and squid. The amino acids were dominated by aspartic and glutamic acids followed by glycine, alanine, leucine and lysine. The composition of free protein-amino acids was dominated by glutamate and threonine, followed by leucine that was particularly high in yeast extract. Yeast extract had the highest content of free amino acids, followed by sand eel hydrolysate and silage from salmon and white fish. All four samples had a high content of threonine and leucine, followed with a mix of different amino acids. Gutted squid had a low level of free protein-amino acids, but more than twice the concentration of the non-protein amino acid taurine, compared to the other attractant solutions (Table 3).

2.3. Experimental design and behavior patterns

The experimental tanks were two outdoors fiberglass tanks (3 m diameter, 7 m^3), with a water depth of 1 m. They had a constant flow of filtered seawater at ambient temperature 9 °C (± 0.6). The experimental tanks were covered by black fine meshed netting (10 mm half mesh) and a tarpaulin cover on the sunny side (see Fig. 2d). Every second day, five fish were randomly picked from the holding tank and transferred to each of the two experimental tanks. The two experimental tanks were placed next to the holding tank, and the fish was netted and carried to the experimental tanks at an air exposure of less than 5 s. The seawater entered the tanks through a pipe close to the tank wall. Perforations along the entire length on one side of the pipe caused a circular current in the tanks. The water outlet was in the center of the tanks (see Fig. 2).

After the fish were acclimatized overnight in the experimental tanks, the nine attractants were presented to the fish in a random order over two consecutive days: five attractants the first day and four the second day. The experiment took place outdoors in November at a latitude of 69 °N, thus, light hours were limiting. Each attractant was injected into the tank in a 250 ml portion over a period of 10 − 12 min by a peristaltic pump through a silicone hose. The mouth of the hose was positioned at 0.5 m depth at the opposite side of the seawater inlet, centered between the outlet and the tank wall. Seawater was always introduced through the silicon hose for 20–30 min prior to introducing of an attractant (both before the first attractant was introduced and in between introductions of attractants). Thus, the time period between introduction of an attractant was 20 − 30 min or 24 h. The introduction of seawater through the silicone hose, together with a high water exchange rate (> 40 l/min), made sure that the attractant was flushed out of the system before a new attractant was introduced. The experimental period lasted for 10 days, resulting in ten experimental runs (replicates) for each attractant.

Fish behavior was recorded by a GoPro camera that was submerged on the opposite side of the tank from where the attractant was introduced. We recorded behavior from 5 min before the attractant was injected until all attractant was introduced (a total recording time of 15 − 17 min). For the behavioral analysis we used the 5 min periods before (control) and after attractant injection started.

Based on the video recordings, five behavior responses were quantified:

Food search behavior:

1) Search; the number of times a fish turned with its barbel touching the bottom of the tank (this is the same behavioral response used in Ellingsen and Døving, 1986).

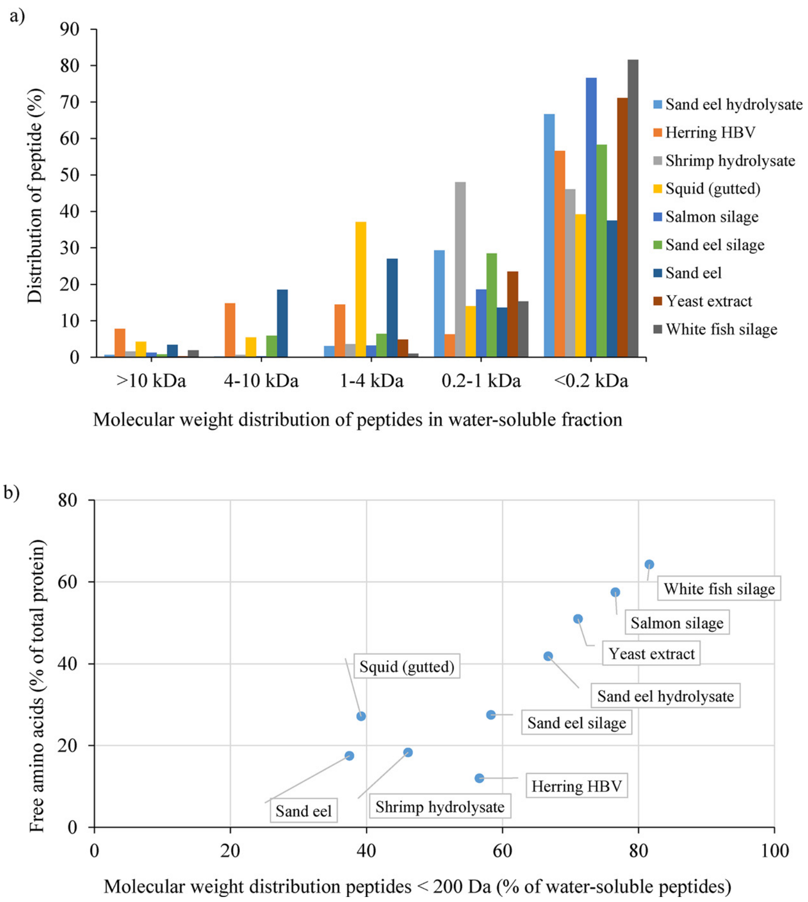

Fig. 1. Molecular weight distribution of peptides in water-soluble fraction of attractants. Distribution of different peptides sizes (a) and correlation between the relative content of small peptides ($<$ 200 daltons (Da)) and free amino acids (g/100 g attractant solution) (b). Herring HBV: herring by-products of head, bone and visceral.

2) Plume; the number of times a fish entered the attractant plume. Plume being defined as the near (within two body lengths) downstream area to the attractant outlet.
3) Approach; the number of times a fish swam upstream towards the attractant outlet approaching the hose at a distance of $<$ 10 cm.

Feeding behavior:

1) Touch; the number of times a fish touched the end of the hose (the last $10-15$ cm) with its mouth, nose or its barbel.
2) Bite; the number of times a fish bit or opened its mouth to grasp the end of the hose (the last $10-15$ cm).

These behaviors were intended to describe a gradient of interest in the attractant. The fish were not tagged in order to minimize potential stressors.

2.4. Statistical analyses

Cod were expected to respond to all tested attractants, given that only known baits and attractant products for cod were tested. Therefore, the aim of the study was to reveal the most efficient of the attractants in terms of triggering food search and feeding behavior. The experimental fish were not individual marked, and we could therefore not control for multiple observations of the same fish. Thus our sample size was 10, one for each groups of fish, and not the individual fish. A paired *t*-test was used to compare the mean behavioral responses between control period (seawater introduced trough the silicone hose) and attractant period (attractant introduced trough the silicone hose). Ranking was used to differentiate between the different attractants. The larger the increase in responses (search, plume, approach, touch, bite) for an attractant compared to during the control period, the better rank (best rank is 1, lowest rank is 9). In addition, we conducted a one-way analysis of variance (ANOVA) with time as a covariate to compare the five behavior responses among the nine attractants (Box et al., 1978). Time was used as covariate since the experiment was conducted over a ten-day period and the cod were not fed until the end of the experiment. The Least Significant Difference (LSD) method was used to form likely homogeneous groups of attractants, i.e. to group attractants that had similar average behavioral responses.

3. Results

In general, search, approach and in plume behavior increased when comparing the 5-min before (control period) and after introduction of an attractant (Fig. 3, paired *t*-test, $P \leq 0.001$, Supplementary Table 2). The greatest increase was observed for approach towards the attractant. Fish biting or touching the hose with the mouth were only observed after attractant was introduced, never during the control periods. The average number of times that approach, search, in plume, touch and bite were observed during the 5-min period after introduction of a given attractant is presented as added bars in Fig. 4, with the according rank presented as white numbers inside the bars (see also Supplementary Table 3). Further, calculation of mean rank ($\bar{x}$ Rank) of each attractant revealed the most stimulating attractants to be sand eel hydrolysate, herring and shrimp hydrolysate (Fig. 4, Supplementary Table 3). These three attractants scored best for approach, bite and touch.

One-way analysis of variance with time as a covariate, showed that average number of approaches combined with number of touches, were more varied than any other (single - or combinations) responses to the nine attractants. In other words, the largest variation between attractants was found in relation to the cod's attraction and touch activity. This was found when controlling for time (using time as a covariant). The probability was $P = 0.056$ that the average number of approaches

Table 3

Total amino acid and free amino acid composition of the tested attractants. AA = amino acids, FAA = free amino acids, n.a. = not analyzed.

Total amino acids and free amino acids	Sand eel hydrolysate		Herring HBV		Shrimp hydrolysate		Squid (gutted)		Salmon silage		Sand eel silage		Sand eel		Yeast extract		White fish silage	
(g/100 g)	AA	FAA	AA	FAA	AA	FAA	AA	FAA	AA	FAA	AA	FAA	AA	FAA	AA	FAA	AA	FAA
Glutamate (-mine; -mic acid)	0.05	0.05	0.09	0.01	0.06	< 0.01	0.06	0.01	0.09	0.03	0.17	0.04	0.08	0.01	n.a.	0.13	0.1	0.03
Serine	0.02	0.01	0.04	< 0.01	0.02	< 0.01	0.02	< 0.01	0.04	0.02	0.06	0.01	0.03	< 0.01	n.a.	0.06	0.04	0.02
Glycine	0.03	0.01	0.04	< 0.01	0.04	< 0.01	0.02	< 0.01	0.06	0.02	0.08	0.01	0.03	< 0.01	n.a.	0.04	0.07	0.02
Histidine	0.01	0.01	0.02	0.01	0.01	< 0.01	0.01	< 0.01	0.02	0.01	0.04	0.01	0.02	0.01	n.a.	0.02	0.02	< 0.01
Threonine	0.03	0.06	0.04	0.02	0.02	0.02	0.02	0.04	0.03	0.04	0.06	0.04	0.03	0.03	n.a.	0.08	0.03	0.06
Alanine	0.04	0.02	0.06	< 0.01	0.03	0.01	0.04	< 0.01	0.05	0.02	0.08	0.01	0.04	0.01	n.a.	0.08	0.06	0.02
Arginine	0.02	0.03	0.05	0.02	0.03	0.01	0.03	0.01	0.04	0.02	0.08	0.01	0.03	0.01	n.a.	0.04	0.05	0.03
Proline	0.03	< 0.01	0.04	< 0.01	0.03	0.01	0.04	0.03	0.04	0.01	0.05	0.01	0.02	< 0.01	n.a.	0.03	0.04	0.01
Tyrosine	0.04	0.02	0.03	< 0.01	0.02	< 0.01	0.02	< 0.01	0.03	0.02	0.05	0.01	0.02	< 0.01	n.a.	0.02	0.03	0.02
Valine	0.06	0.02	0.05	< 0.01	0.03	< 0.01	0.02	< 0.01	0.04	0.02	0.07	0.01	0.04	< 0.01	n.a.	0.07	0.04	0.02
Methionine	0.04	0.03	0.02	< 0.01	0.01	< 0.01	0.01	< 0.01	0.02	0.03	0.04	0.03	0.02	0.01	n.a.	0.05	0.02	0.03
Isoleucine	0.06	0.02	0.04	< 0.01	0.02	< 0.01	0.02	< 0.01	0.03	0.02	0.06	0.01	0.03	< 0.01	n.a.	0.07	0.03	0.03
Leucine	0.09	0.05	0.07	< 0.01	0.03	0.02	0.03	0.01	0.06	0.05	0.09	0.04	0.05	0.01	n.a.	0.11	0.05	0.04
Lysine	0.09	0.04	0.08	0.01	0.04	< 0.01	0.04	< 0.01	0.07	0.03	0.11	0.02	0.07	0.01	n.a.	0.06	0.06	0.04
Sum protein AA	***0.71***	***0.42***	***0.77***	***0.07***	***0.43***	***0.08***	***0.44***	***0.1***	***0.72***	***0.4***	***1.18***	***0.29***	***0.6***	***0.1***	***n.a.***	***1.01***	***0.76***	***0.4***
Creatinine		0.03		0.02		0.02		0.02		0.03		0.03		0.02		0.04		0.03
Taurine		0.01		0.03		0.01		0.07		0.02		0.01		0.02		< 0.01		0.02
4-aminobutanoic acid		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		0.01		< 0.01
Anserine		< 0.01		< 0.01		< 0.01		< 0.01		0.01		< 0.01		< 0.01		< 0.01		< 0.01
Ornithine		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		0.01		< 0.01
Sum non-protein AA		***0.04***		***0.05***		***0.03***		***0.09***		***0.06***		***0.04***		***0.04***		***0.06***		***0.05***
SUM FAA		0.46		0.12		0.11		0.19		0.46		0.33		0.14		1.07		0.45

and touches combined - versus attractants were equal. The probability that time had no effect was P = 0.038. Further, LSD method (at the 80 % level) showed that the three attractants with the highest LS mean were sand eel hydrolysate, herring and shrimp hydrolysate (Table 4). In other words, the cod were showing the highest attraction and touch activity towards sand eel hydrolysate, herring HBV and shrimp hydrolysate. These were the same three attractants that came out best in the rank analysis (Fig. 4).

The three products made from sand eel showed that processing of the raw material to silage and hydrolysates increased the content of free amino acids and stimulated a higher frequency of approach, bite and touch towards the attractant ($R^2 = 0.99$, Fig. 5a). However, no such correlation ($R^2 = 0.34$) was found for the other attractants (Fig. 5b). Herring and shrimp hydrolysate, which were overall the best-ranked attractants, had the lowest content of free amino acids (Fig. 5b). Shrimp hydrolysate, however, had the highest content (35 %) of small peptides (200 − 1000 Da), while herring had the second highest content of taurine after squid (Table 3), which indicate that also small peptides and taurine may be important for attractants.

4. Discussion

This study showed that all attractants tested triggered wild Atlantic cod to increase their food search (approach, plume and search) and feeding behavior (bite and touch). The results indicate that there are several by-products from the aquaculture and fisheries industries that have a potential as an attractant for artificial longline and pot baits for cod. The three most effective attractants were herring, sand eel hydrolysate and shrimp hydrolysate, which all elicited a higher food search and feeding behavior than squid that was used as reference. Thus, these three attractants should have the greatest potential for incorporation in a bait matrix (manufactured bait) to be tested in commercial fishing.

Many studies have attempted to identify the chemical nature of feeding attractants and stimulants in teleost fishes (see review by Hara, 1992, 2011; Kasumyan and Døving, 2003). These studies have demonstrated that amino acids or water-soluble extracts have the most stimulatory capacity to elicit feeding behavior in fish (Marui and Caprio, 1992; Friedrich, 2006; Hara, 2011), and studies have shown that food extracts lose their stimulating effects when their amino acids have been eliminated (Carr and Derby, 1986). However, each fish species selectively responds to a specific mixture of compounds, and synthetic mixtures of amino acids seldom attain the effectiveness of the extracts from natural foods (Carr and Derby, 1986; Ellingsen and Døving, 1986; Jones, 1992; Hara, 2011). Thus, we tested aqueous extracts of natural marine resources. Our study demonstrated a relationship between stimulatory capacity and concentration of free amino acids for the three attractants based on sand eel, indicating that free amino acids are important feeding attractants for cod (Fig. 5a). Studies designed to identify the active components in natural shrimp and squid extracts confirmed that amino acids were the major feeding stimulants for cod (Ellingsen and Døving, 1986; Johnstone and Mackie, 1990).

However, a relationship between stimulatory capacity and concentration of amino acids was not seen for the other attractants tested in this study (Fig. 5b). Although amino acids are shown to be important feeding attractants for cod (Ellingsen and Døving, 1986), these findings demonstrate that other compounds than single amino acids are important in eliciting feeding responses in cod. Furthermore, the complexity of protein/peptide composition of amino acids make it difficult to determine the effect of single amino acids in aqueous extracts. Thus, attempts to identify efficient feeding attractants to be incorporated in alternative baits should be based on using complete aqueous extracts, rather than incorporating a mixture of single components that are shown to play a major role in stimulating feeding behavior.

The most potent attractant solutions tested were herring, sand eel hydrolysate and shrimp hydrolysate, which were more efficient in

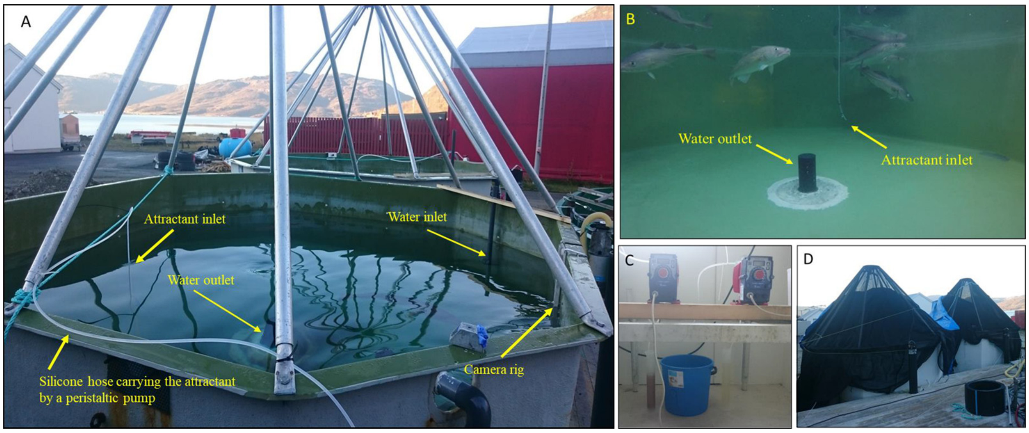

Fig. 2. Experimental design. The behavioral study was conducted in two fiberglass tanks (3 m diameter, water depth 1 m ~ 7 m^3 of water) (A). Seawater entered the tanks through a perforated pipe close to the tank wall. The water outlet was in the in the center of the tanks. The attractants were introduced to the tanks by a peristaltic pump (C) through a silicone hose, which outlet was positioned at 0.5 m depth at the opposite side of the seawater inlet (A and B). Picture (C) shows the peristaltic pumps (two as two experiments were run parallelly in the two tanks) pumping the attractant from 250 ml measuring cylinders. Picture (D) shows the two experimental tanks during the experiment, when they were all covered with netting (10 mm half mash) and tarpaulin shading on the sunny side.

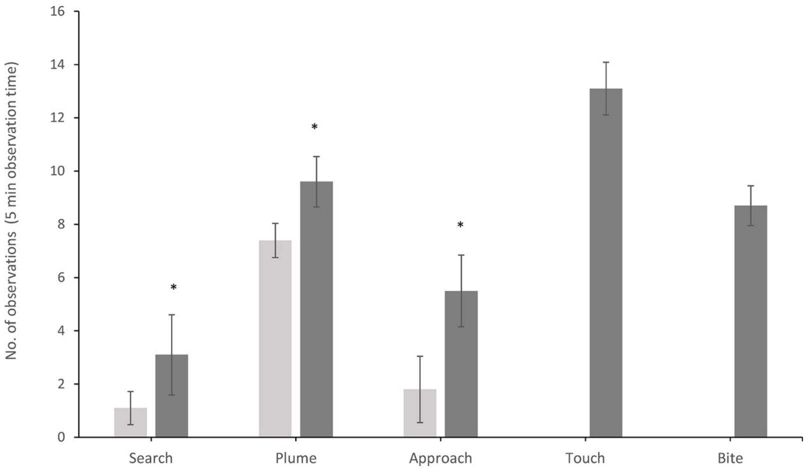

Fig. 3. Results from all attractants combined. Behavioral response of cod during control (gray bars) and after introduction of attractant (dark grey bars). The cod never bit or touched the introducing hose during the control periods. Arrow bars represents standard variation, and star (*) pairwise *t*-test with a significance of $P \leq 0.001$.

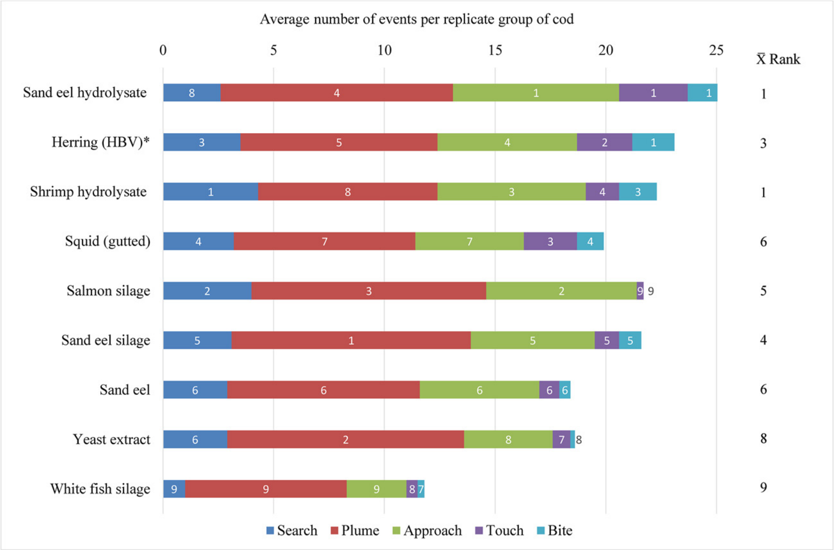

Fig. 4. The responsiveness of cod to the different attractants. This figure shows the average number of times the five behavioral responses (approach; search; bite; touch and in plume) were observed during introduction of the different attractant as added on bar values. The attractants are ranked for which caused the highest average response. Rank number is given as white numbers within the bar. The highest average is ranked 1, lowest 9. The average rank ($\bar{x}$ Rank) for each attractant across the five behavioral responses is presented in a column to the right. This average rank is used as a measure of the responsiveness of the cod to the different attractants.

eliciting feeding responses in cod compared to the attractant based on squid. In a hydrolysis process, proteins are cleaved into smaller and more water-soluble peptides and free amino acids, and in this form, they are more likely to stimulate the feeding behavior in fish, as compared to the non-processed protein substrate. In this study, this was shown for the sand eel substrate with best response given for the hydrolysis process, followed by silage and non-processed. The free amino acids content was high for the sand eel hydrolysate, but low for the shrimp hydrolysate and herring. Thus, also this result indicates that compounds other than the free amino acids are important in eliciting feeding responses in cod. However, for shrimp hydrolysate, the high content of small peptides (200 – 500 Da) with two or more amino acids could have similar stimulatory effect as free amino acids, while for herring a high content of taurine was observed. Squid with the highest

Table 4

The least-square mean of the number of approaches and touches by cod for each attractant. The estimates were based on an ANOVA with time as a covariate. The grouping of the attractants was based on the LSD method, where different letters indicate statistical difference at the 80 % level.

Attractant	LS Mean	Homogeneous Groups
Sand eel hydrolysate	10.6	A
Herring	8.9	AB
Shrimp hydrolysate	8.1	AB
Squid	7.3	BC
Salmon silage	7.2	BC
Sand eel silage	6.7	BC
Sand eel	6.3	BC
Yeast extract	4.8	CD
White fish silage	3.1	D

content of taurine showed moderate behavioral responses.

5. Conclusions

The main objective of this bioassay study was to identify potent marine resources to be incorporated in a manufactured bait that can replace traditional baits used to target cod. Our approach is cost-effective compared to full-scale fishing experiments that require vessel time and the challenging process of formulating a bait matrix (binder) to hold and effectively release the attractants. All attractants tested triggered feeding responses in cod, indicating that there are several by-products from the fishing industry that has potential as an attractant for an artificial longline bait. Although our results did not produce pronounced and significantly different behavioral responses in cod towards the attractant solutions tested, the three products that ranked highest (herring processing by-products, sand eel hydrolysate and shrimp hydrolysate) were at least as efficient as squid which fishermen claim to be the best bait for catching cod. These products are available in large quantities, at relative low prices and not used for human consumption. Thus, we have identified potential marine resources that may prove to form the basis as attractants for an efficient manufactured bait.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by a grant from The Research Council of Norway (NFR). We like to thank Sten Ivar Siikavuopio, Tor Evensen and

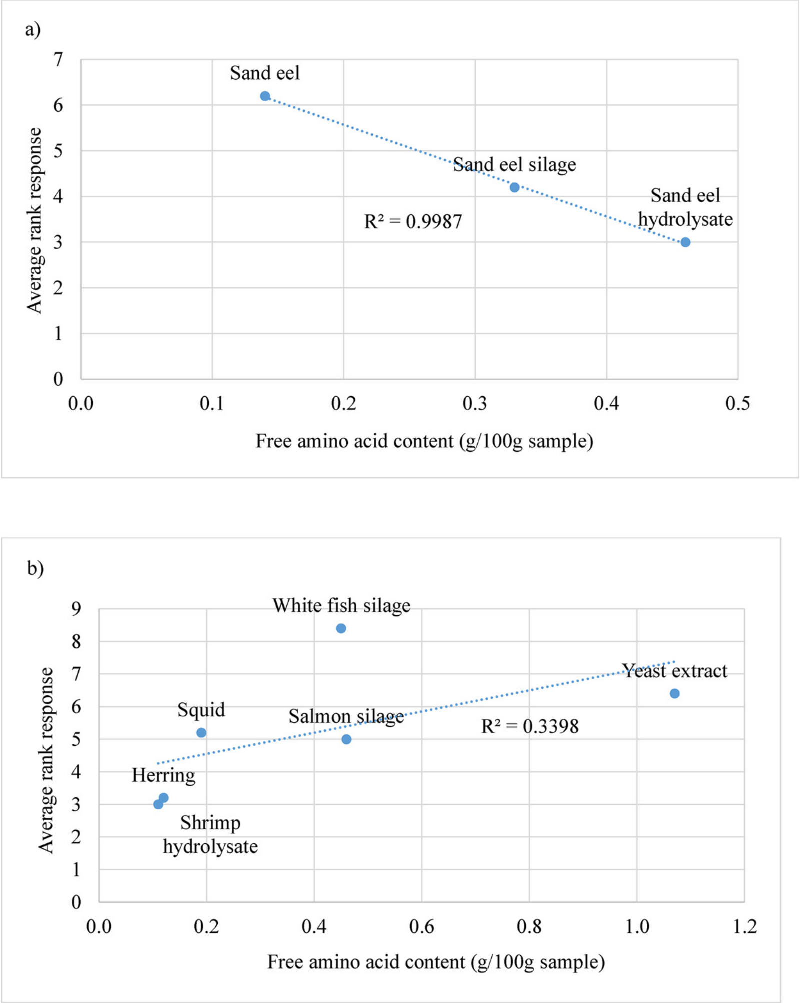

Fig. 5. Responsiveness of fish related to amount of free amino acids in attractant. These two figures are comparing amount of free amino acids in an attractant - with the cods' average responsiveness to the same attractant. The correlation for the sand eel attractants is shown in (a) and for the rest of the attractants in (b). As a measure of the cods' responsiveness we have used the *average rank* values given in last column in Figure 4, thus, *Average rank response* on the y-axis. The lower the rank number the higher response to the attractant.

Tina Thesslund at Nofima for providing live caught cod - and for their assistance and guidance in relation with the cod behavioral experiments performed at Nofima's laboratory in Kårvika, Tromsø, Norway.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fishres.2020.105535.

References

Ageeva, T.N., Jobling, M., Olsen, R.L., Esaiassen, M., 2017. Gender-specific responses of mature Atlantic cod (Gadus morhua L.) to feed deprivation. Fish. Res. 188, 95–99.

Bidlingmeyer, B.A., Cohen, S.A., Tarvin, T.L., Frost, B., 1987. A new, rapid, high-sensitivity analysis of amino acids in food type samples. J. Assoc. Off. Anal. Chem. 70, 241–247.

Bjordal, A., Løkkeborg, S., 1996. Longlining. Fishing News Books, Oxford, pp. 156.

Box, G.E.P., Hunter, W.G., Hunter, J.S., 1978. Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building. Wiley, New York, NY p 653.

Carr, W.E.S., Derby, C.D., 1986. Chemically stimulated feeding behavior in marine animals. J. Chem. Ecol. 12, 989–1011.

Ellingsen, O.F., Døving, K.B., 1986. Chemical fractionation of shrimp extracts inducing bottom food search behavior in cod (Gadus morhua L.). J. Chem. Ecol. 12, 155–168.

FAO, 2018. In: The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

FAO, IFAD, WFP, 2015. The state of food insecurity in the world 2015. In: Meeting the 2015 International Hunger Targets: Taking Stock of Uneven Progress. Rome, FAO.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation andpurification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Frid, C.L.J., Paramor, O.A.L., 2012. Feeding the world: what role for fisheries? ICES J. Mar. Sci. 69, 145–150.

Friedrich, R.W., 2006. Mechanisms of odor discrimination: neurophysiological and behavioral approaches. Trends Neurosci. 29, 40–47.

Hara, T.J., 1992. Overview and introduction. In: Hara, T.J. (Ed.), Fish Chemoreception. Fish and Fisheries Series 6. Chapman & Hall, London, pp. 1–12.

Hara, T.J., 2011. Smell, taste and chemical sensing. In: In: Farrell, A.P. (Ed.), Encyclopedia of Fish Physiology: From Genom to Environment, vol 1. Academic Press/Elsevier, London/ Amsterdam, pp. 183–235.

Henriksen, E., 2009. Det egner seg! Nofima report no. 28/2009 (in Norwegian).

Humborstad, O.-B., Mangor-Jensen, A., 2013. Buoyancy adjustment after swimbladder puncture in cod *Gadus morhua*: an experimental study on the effect of rapid decompression in capture-based aquaculture. Mar. Biol. Res. 9, 383–393. https://doi.org/10.1080/17451000.2012.742546.

Humborstad, O.-B., Davis, M.W., Løkkeborg, S., 2009. Reflex impairment as a measure of vitality and survival potential of Atlantic cod (*Gadus morhua*). Fish. Bull. 107, 395–402.

Humborstad, O.-B., Breen, M., Davis, M.W., Løkkeborg, S., Mangor-Jensen, A., Midling, K., Olsen, R.E., 2016. Survival and recovery of longline- and pot-caught cod (*Gadus morhua*) for use in capture-based aquaculture (CBA). Fish. Res. 174, 103–108.

ISO 5983-2, 2009. Animal Feeding Stuffs – Determination of Nitrogen Content and Calculation of Crude Protein Content – Part 2: Block Digestion and Steam Distillation Method. International Organization for Standardization, Geneva, Switzerland.

ISO 6496-2, 1999. Animal Feeding Stuffs - Determination of Moisture and Other Volatile Matter Content. International Organization for Standardization, Geneva, Switzerland.

Januma, S., Miyajima, K., Abe, T., 2003. Development and comparative test of squid liver artificial bait for tuna longline. Fish. Sci. 69, 288–292.

Johnstone, A.D.F., Mackie, A.M., 1990. Laboratory investigations of bait acceptance by the cod, (Gadus morhua L.): identification of feeding stimulants. Fish. Res. 9, 219–230.

Jones, K.A., 1992. Food search behavior in fish and the use of chemical lures in commercial and sports fishing. In: Hara, T.J. (Ed.), Fish Chemoreception. Chapman & Hall, London, pp. 288–320.

Kasumyan, A.O., Døving, K.B., 2003. Taste preferences in fishes. Fish Fish. 4, 289–347.

Klemetsen, A., 1982. Food and feeding habits of cod from the Balsfjord, northern Norway during a one-year period. ICES J. Mar. Sci. 40, 101–111. https://doi.org/10.1093/icesjms/40.2.101.

Kristinsson, H., 2007. Aquatic food protein hydrolysates. In: Shahidi, F. (Ed.), Maximising the Value of Marine By-Products. Woodhead, Cambridge, pp. 229–247.

Liu, H.J., Chang, B.Y., Yan, H.W., Yu, F.H., Liu, X.X., 1995. Determination of amino acids in food and feed by derivatization with 6-aminoquinolyl-N-hydroxysuccinimedyl carbamate and reversed-phase liquid chromatographic separation. J. AOAC Int. 78 (3), 736–744.

Løkkeborg, S., 1998. Feeding behavior of cod, (Gadus morhua): activity rhythm and chemically mediated food search. Anim. Behav. 56, 371–378.

Løkkeborg, S., 2013. Agnforbruket i det Norske linefisket: En analyse av årsakene til variasjonen i anvendelsen av de ulike agntypene. Rapport fra Havforskningen Nr. 24-2013. pp. 13 (in Norwegian). .

Løkkeborg, S., Fernö, A., 1999. Diel activity pattern and food search behavior in cod, *Gadus morhua*. Environ. Biol. Fish 54, 345–353.

Løkkeborg, S., Siikavuopio, S.I., Humborstad, O.-B., Utne-Palm, A.C., Ferter, K., 2014. Towards more efficient longline fisheries: fish feeding behaviour, bait characteristics and development of alternative baits. Rev. Fish Biol. Fish. 24, 985–1003.

Marui, T., Caprio, J., 1992. Teleost gustation. In: In: Hara, T.J. (Ed.), Fish Chemoreception. Fish & Fisheries Series, vol 6 Springer, Dordrecht.

Pol, M.V., Correia, S.J., MacKinnon, R., Carver, J., 2008. Longlining haddock with manufactured bait to reduce catch of Atlantic cod in a conservation zone. Fish. Res. 94, 199–205.

Raa, J., Gildberg, A., 1982. Fish silage: a review. Crit. Rev. Food Sci. Nutr. 16 (4), 383–419.

Siikavuopio, S.I., James, P., Stenberg, E., Evensen, T., Sæther, B.S., 2017. Evaluation of protein hydrolysate of by-product from the fish industry for inclusion in bait for longline and pot fisheries of Atlantic cod. Fish. Res. 188, 121–124.

Wang-Andersen, J., Haugsgjerd, B.O., 2011. Forbedret analysemetodikk for peptidstørrelsesfordeling i marine proteinhydrolysat. Nofima report 43. 30 pp. (in Norwegian). .

Winther, et al., 2013. Sektor analyse for de marine næringene i Nord-Norge-statusbeskrivelse og fremtidsutsikter. SINTEF Fiskeri og havbruk AS, A24298, Report 2013. 212 pp. (in Norwegian). .