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RESEARCH

# Marine allergens in farmed seaweed: considerations for precautionary labelling

**Jennifer Mildenberger**[1] · **Céline Rebours**[1]

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## Abstract

Seaweed aquaculture is growing and an increasing number of seaweed products is introduced on the food market. Contamination by marine allergens is a concern for the food industry and recommendations are missing on if and how products need to be labelled to assure food safety. Two species of kelp were sampled from four farms along the Norwegian coastline during two consecutive years. The samples were quantified for their content of crustacean and mollusc tropomyosin and fish parvalbumin by commercial ELISA (Enzyme-Linked Immunosorbent Assay) kits. All three seafood allergens were detected in several kelp samples with high variation, but without a specific pattern. We also studied samples in relation to their location within one farm, seeding methods, algae parts and a shifted harvesting period, with some aspects leading to differences. Samples were also analysed after blanching and fermentation at one studied farm and drying and powdering at a food processor. No major changes in allergen levels were observed after blanching and fermentation, but sample numbers might have been limiting. Homogenisation in larger quantities led to less variation between replicates, however, cross-contamination needs to be avoided. Detected marine allergens in the studied samples were below critical levels associated with an allergenic risk and would not require labelling according to the widely used VITAL (Voluntary Incidental Trace Allergen Labelling) guideline. However, this consideration is the responsibility of the food producer and needs to be based on analyses done for each batch of products as part of the general food safety evaluation.

**Keywords** Seaweed · Diet · Food safety · *Alaria esculenta* · *Saccharina latissima* · ELISA

## Introduction

In the last 20 years kelp aquaculture has developed in Europe (Avitabile et al. 2023) with most of the biomass being processed as ingredients for the food industry (Vazquez Calderon and Sanchez Lopez 2022). Thus, much work has been done to quantify potentially harmful elements in macroalgae such as iodine, arsenic and heavy metals (Roleda et al. 2019; Jordbrekk Blikra et al. 2021; Krook et al. 2024). There is still limited data on the occurrence of marine allergens in seaweed and the significance of risk to consumers through the consumption of seaweed or seaweed-based foods (Banach et al. 2020; Mildenberger et al. 2021). Today, no Codex standard or guidelines exist to specifically address food safety vis-à-vis seaweed production, processing and consumption, and FAO and WHO reported that there is a significant global regulatory gap concerning food safety in seaweed (FAO & WHO 2022). However, it is the obligation of food producers to establish a food safety management system assuring safe and, in terms of allergens, correctly labelled food products. For the inclusion of kelp as for any other ingredient included in existing productions, knowledge on the allergen status is essential to feed into allergen management routines (Allergen Bureau 2023).

In fact, allergenic components receive increased attention as allergies are more frequently reported (Loh and Tang 2018; Eisenstein et al. 2020) with a slight increase from 2.6 % for the 2000 – 2012 period to 3.5 % for the 2012–2021 period in Europe (Spolidoro et al. 2023). Yet scarce, some studies have reported an allergenic potential of algae (Polikovsky et al. 2019; Thomas et al. 2019; Banach et al. 2020). James et al. (2023) reviewed allergenic reactions in humans after ingestion of algae and reported mainly allergenic

✉ Jennifer Mildenberger
jennifer.mildenberger@moreforsking.no

✉ Céline Rebours
celine.rebours@moreforsking.no

[1] Møreforsking AS, Borgundvegen 340, 6009 Ålesund, Norway

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reactions on microalgae, red macroalgae or extracted carrageenan. More readily assessed, allergenic proteins from other marine organisms may unintentionally be included or accumulated in seaweed products from the environment or during processing. Farm infrastructure or the macroalgae themselves are known to be adequate substrates for settlement of shellfish larvae or habitat for a variety of fish species (Fitridge et al. 2012; Visch et al. 2020). Consequently, allergens of marine species, such as shellfish and fish have already been reported to find their way into the production line (Motoyama et al. 2007; Mildenberger et al. 2021). The contamination of seaweed ingredients by marine allergens is therefore a critical aspect to control and document when producing seaweed-based ingredients for human consumption.

Seafood allergens are an important concern in food production (Dhruve et al. 2024). Fish is considered to be responsible for 90 % of food allergic reactions and is often a lifelong clinical allergy, while crustacean allergies have been less reported and might decrease with age. Adverse reactions to molluscs might also be caused by infectious agents or toxins or are not clearly distinguished from crustacean allergies and no study has yet investigated their time trends (EFSA NDA Panel 2014). In fish, one of the most relevant allergenic proteins is parvalbumin, while crustacean tropomyosin is an important allergen in crustaceans such as shrimp, lobster or crab. In molluscs like mussels, scallops or squid, mollusc tropomyosin is among the known allergens (Ruethers et al. 2018).

Precautionary Allergen Labelling (PAL) has globally become the most common practice to provide consumer information about the unintended allergen presence (UAP) in food products. However, some countries prohibit the use of PAL, while others have encouraged the labelling of products with various statements and advised different reference doses (RfDs) for allergen management. These non-uniform and indiscriminate practices impact the hazard assessment, and trade of the products and are not yet effective in communicating the risk to consumers and ought to be improved (Madsen et al. 2020; FAO & WHO 2023). The European food regulation imposes the mandatory labelling of specified allergenic foods including fish, crustaceans and molluscs, when used or added to food. Article 36 also states that voluntary information should not be misleading, or ambiguous and should be based on scientific data, but no indication of RfDs is given in the European food regulation (Regulation (EU) n° 1169/2011 2011).

The VITAL® (Voluntary Incidental Trace Allergen Labelling) approach was therefore developed over time to establish a program of risk-based PAL (Allergen Bureau 2023). The latest update of this program adopted the eliciting dose (ED) $ED_{01}$ as RfD. The ED is derived from a stacked model averaging program for each of the 14 European Union food allergens. $ED_{01}$ corresponds to the amount of total protein from an allergenic food below which only 1 % of the allergic population would react with objective allergic symptoms (VITAL Scientific Expert Panel 2019). The exposure to allergenic protein in a certain food product depends further on a reference amount (RfA), which is the maximum amount of food eaten on a typical eating occasion. To define the labelling decision for packaged foods, Action Levels (AL) have been defined. AL 1 corresponds to a low concentration of the evaluated allergen with a low risk for adverse reaction and does not recommend PAL. AL 2 corresponds to a significant concentration of the evaluated allergen and requires PAL (depending on the legislation in the country of manufacture or sale). The AL transition point is the amount of allergenic protein in the food product from which the intake of one standard portion (the RfA) results in exposure to the RfD, possibly causing an allergic reaction in allergic consumers (Allergen Bureau 2023; FAO & WHO 2023).

The VITAL 3.0 panel recommends 25 mg of shrimp protein and 1.3 mg of finfish protein as RfDs for crustaceans and fish, respectively (VITAL Scientific Expert Panel 2019). Generally, mollusc and crustacean allergies are easily confused and are often commonly described as shellfish allergy (Rolland et al. 2018). Thus, specific recommendations for molluscs are still not available. These marine allergens can be detected in dried seaweed samples by commercial ELISA (Enzyme-Linked Immunosorbent Assay) kits (Mildenberger et al. 2021). ELISA is based on the direct recognition of a target protein by specific antibodies and visualization of the reaction by a proportional change in absorbance. ELISA is well established for the detection of seafood allergens with the advantage of direct detection by binding the allergenic protein (Dhruve et al. 2024).

Thus, there is a need to better characterize the presence of unintended allergens in different species of commercially produced macroalgae to be able to determine the related food safety risk. Norway is the largest producer of macroalgae in Europe with 111 sites qualified for seaweed production, where the kelp species *Saccharina latissima* and *Alaria esculenta* take an important share of attributed licenses (Directorate of fisheries 2024). Thus, our study selected four seaweed farms along the Norwegian coastline to assess variations in the presence of marine allergens between farms, species and production years as well as in relation to locations within one farm, the seeding method, the part of the seaweed being harvested and a shifted harvesting period.

Further, little information is available to understand how the content of the three marine food allergens in seaweed is affected during the processing in the manufacturing facility. Reports on allergenicity changes in processed food describe both increasing and decreasing effects. Likewise, crustacean allergens were reported to be increased after thermal processing (Laly and Sankar 2021), while fermentation was

reported to reduce crustacean tropomyosin (Amalia et al. 2023). Therefore, samples of kelp that was blanched and fermented by one studied farm and dried and powdered by a food processor were also analysed to document potential changes in allergenic proteins during this processing.

## Materials and methods

### Macroalgae sample collection and preparation

Fresh macroalgae were collected from four farms dedicated to kelp culture located along the Norwegian West coast. The locations of the farms are shown on the map of Norway (Fig. 1). Samples were either collected manually shortly before or during commercial harvesting. If sampled before harvesting time, the kelp was fully pulled from the ropes (holdfast, stipe and blade), while kelp samples collected during harvesting were cut above the holdfast. Samples always contained several macroalgae individuals, packed as 1 kg in a plastic bag per sampling location. Farm 1 (F.1) also collected samples one month after the usually practiced harvesting time, when maximal biomass with the least fouling can be obtained at this farm. The seaweed producers were also asked to provide information about farm characteristics, seaweed status and marine organisms that were observed visually for both collection years. The noted observations of marine organisms were simplified to indicate the presence or absence of fish, crustaceans and molluscs and were arranged in relation to ELISA results (Supplementary Table 1). Collected samples were frozen and sent to Møreforsking AS and stored at −20 ˚C until further processing. The frozen samples were chopped into pieces and 100 g from different parts were freeze-dried and homogenized in an IKA Tube Mill C S000. In 2023, the samples from F.3 and F.4 were likewise prepared and freeze-dried at the Institute of Marine Research (NO-5817 Bergen) and sent to Møreforsking for analysis. Analysis of samples was done as soon as possible after drying and concluded for all samples before the next season.

F.3 has a processing line for blanching and fermenting of kelp, from which 5 samples of *A. esculenta* were taken for

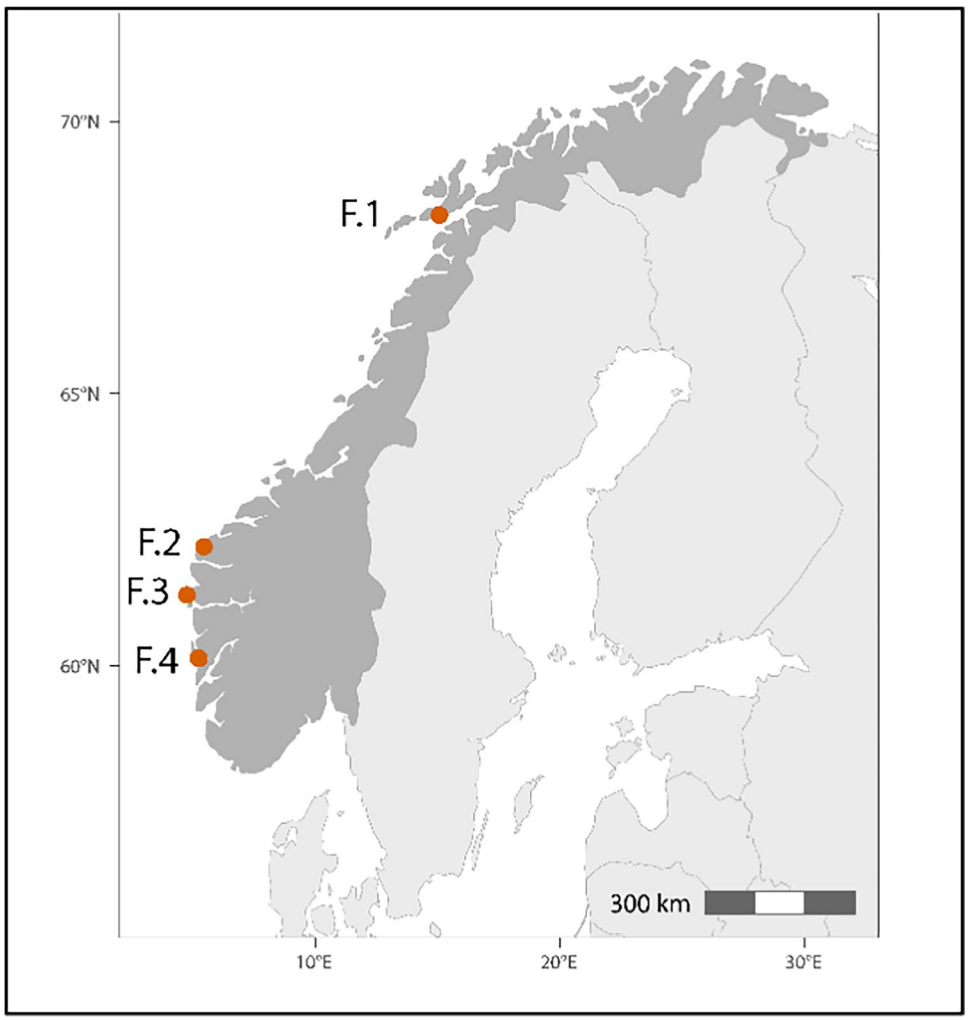


**Fig. 1** Location of the farms (F.1 to F.4 from North to South) on the West coast of Norway from which samples were obtained. More information about the farms is given in Tables 1 and 2

**Table 1** Farms including all cultivation sites, their cultured species and samples collected in 2022 that were used in this study. Production quantities are presented as wet weight (WW). Sample collection before or during harvesting is indicated as field (whole) or harvest (cut), respectively

| | Farm 1 (F.1) | | | | Farm 2 (F.2) | | Farm 3 (F.3) | Farm 4 (F.4) |
|---|---|---|---|---|---|---|---|---|
| Farm name | Lofoten Blue Harvest | | | | Tango Seaweed | | Arctic Seaweed | Ocean Forest |
| **Size (ha)** | 0.06[a] | | | | 2 | | 1.5 | 19.6 |
| **Species** | *A. esculenta* | | *S. latissima* | | *A. esculenta* | *S. latissima* | *A. esculenta* | *S.latissima* |
| **Seeding method** | twine | | twine | | direct | direct | direct | twine |
| **Production (WW, tonne)** | N/A[a] | | N/A[a] | | 2.2 | 2.6 | 64 | 230 |
| **Deployed (meter)** | 500 | | 500 | | 2 160 | 900 | 32 000 | 46 000 |
| **Produced kg (WW) $m^{-1}$** | N/A[a] | | N/A[a] | | 1 | 2.8 | 2 | 5 |
| **Sample collection** | field (whole) | | field (whole) | | field (whole) | field (whole) | harvest (cut) | field (whole) |
| **Sample collection date** | 3 Jun | 10 Jul | 03 Jun | 10 Jul | 30 May | 30 May | 29 Apr | 21 Apr −04May |
| **Number of samples (n)** | 3 | 3 | 3 | 3 | 3 | 5 | 5 | 8 |

[a]Experimental culture on a loop of rope (100 x 6 m), no production

**Table 2** Farms including all cultivation sites, their cultured species and samples collected in 2023 that were used in this study. Production quantities are presented as wet weight (WW). Sample collection before or during harvesting is indicated as field (whole) or harvest (cut), respectively

| | Farm 1 (F.1) | | Farm 2 (F.2) | | Farm 3 (F.3) | | | Farm 4 (F.4) | |
|---|---|---|---|---|---|---|---|---|---|
| Farm name | Lofoten Blue Harvest | | Tango Seaweed | | Arctic Seaweed | | | Ocean Forest | |
| Size (ha) | 0.06[a] | | 2 | | 3 | | | 19.6 | |
| Species | *A. esculenta* | *S. latissima* | *A. esculenta* | *S. latissima* | *A. esculenta* | *S. latissima* | | *A. esculenta* | *S. latissima* |
| **Seeding method** | twine | twine | direct | twine | direct | direct | | twine | twine |
| **Production (WW, tonne)** | N/A[a] | N/A[a] | 4.9 | 6.1 | 42 | N/A[b] | | 8 | 220 |
| **Deployed (meters)** | 375 | 375 | 3 060 | 2 160 | 42 000 | 10 000 | | 5 000 | 43 000 |
| **Produced kg (WW) $m^{-1}$** | N/A[a] | N/A[a] | 1.64 | 2.9 | 1 | N/A[b] | | 1.2 | 5.1 |
| **Sample collection** | field (whole) | field (whole) | field (whole) | field (whole) | harvest (cut) | harvest (cut) | stipes | harvest (cut) | harvest (cut) |
| **Sample collection date** | 5 Jun | 5 Jun | 11 May | 11 Jun | 2 May | 2 May | 2 May | 4 −5 May | 28. Apr −6 May |
| **Number of samples (n)** | 3 | 3 | 10 | 8 | 10 | 10 | 10 | 10 | 3 |

[a]Experimental culture, no production

[b]Final production numbers are not available

each processing step in 2022 and 2023 and 3 samples of *S. latissima* in 2023. The processing is part of Arctic Seaweed's intellectual property, thus reported details are limited. The biomass was prepared as described in Banach et al. (2024). The freshly harvested seaweeds were pre-washed with seawater, blanched for 60 s in 45 °C seawater (in 2023, acid was added at the blanching step) and a fermentation inoculum was added for 3 days at ambient temperature. Either freshly harvested (raw) or blanched and fermented biomass, has further been dried to flakes or powdered in batches of 200 – 300 kg by the food processor Orkla Foods Europe and 5 samples of 200 g were taken from one batch of each type of studied product. For powdered *A. esculenta*, each of the 5 samples was analysed in 5 extracts, while for all other product types, 3 extracts of 4 samples were analysed. Replicates of flake samples were taken out in amounts representing a portion of 1.5 g, as based on current inclusion rates at the food processor (I. M. Birkeland, personal communication, 03 Oct 2023) and were homogenized separately before analysis. *A. esculenta* samples, which were available from corresponding years from both producer and food processor were analysed in the following sequences:

2022: raw, dried, and powdered
2023: raw, blanched, fermented, dried, and powdered

## Dry weight

The dry weight (DW) of the freeze-dried seaweed samples was determined according to NMKL Method No.23, 3rd Ed, 1991 "Moisture and ash, gravimetric determination in meat and meat products". Three replicates of 5 g homogenized sample (exact weight, $W_1$) were dried in an evaporating dish (weight $W_e$) at 102–103˚C overnight until constant weight was obtained. The samples were cooled in a desiccator and weighted ($W_2$). DW was calculated as DW (%) = $(W_2\text{-}W_e)*100/W_1$. All results were adjusted for the DW of the samples.

## ELISA for marine allergens

For the assessment of potential allergens in seaweed, ELISA kits for the antigens fish parvalbumin, mollusc tropomyosin or crustacean tropomyosin (DEFISE1, DEMOLE1 and DECRUE1; Demeditec Diagnostics GmbH, Kiel) were used as previously described, with slight modification (Mildenberger et al. 2021). Freeze-dried and ground seaweed was dissolved in extraction buffer at 10 mg $L^{-1}$ and incubated at 40˚C for 15 min. For the 2022 samples, three extracts of 0.1 g macroalgae were tested and pooled for further analysis, while in 2023 one extract of 0.3 g macroalgae was used in the analysis. The samples were centrifuged (2000 × *g*; 10 min) and the resulting extract was either used directly (fish parvalbumin assay) or 2 times diluted (tropomyosin assays) to further limit matrix effects. Absorption was read at 450 nm in a Synergy HTX S1LFA plate reader. Results were estimated based on 4-parameter logistic regression curves (Arigo Biolaboratories 2022) and final values were adjusted to the dry weight (DW) of the samples. Detection ranges of the allergen assays and limit of quantification (LOQ) for the applied extraction conditions are shown in Table 3. Due to the unavailability of a pure parvalbumin standard, results of the fish allergen assay are given as cod equivalents, although specific for parvalbumin.

## Calculation of AL transition points

To be able to compare ELISA results (given in amount of allergen) to VITAL recommendations (RfDs) (given as amount of allergenic protein), ELISA results were converted (Allergen Bureau 2023). Factors used for the conversion are shown in Table 4. Blue mussel was chosen as a conversion organism for the mollusc assay, as it is the most likely contaminating mollusc in seaweed, but there is no VITAL recommendation available for mollusc.

AL transition points were calculated by the formula: AL transition point *(ppm) = RfD (mg) x (1000/RfA (g)) (Allergen Bureau 2023), where we assumed 3 g as the maximum portion size RfA.

## Statistical analysis

Statistical analyses were performed, and graphs created in GraphPad Prism 10.0.1. One- or two-way ANOVA (or mixed-effects analysis in case of missing values) with

**Table 3** Detection ranges of the allergen assays and limit of quantification (LOQ) in the seaweed samples for the applied extraction conditions provided by Demeditec Diagnostics GmbH. The manufacturer recommends using the LOQ as reliable limit of detection

| Assay target | Assay range (ppm) | Final dilution | LOQ (ppm, in seaweed (DW)) |
|---|---|---|---|
| Crustacean tropomyosin | 0.02 - 0.4 | 10 | 0.2 |
| Mollusc tropomyosin | 0.01 - 0.4 | 10 | 0.1 |
| Fish (cod equivalents) | 4 - 100 | 5 | 20 |

**Table 4** Factors and formulas used for conversion between assay targets and organism protein. Completed with values from a: Demeditec Diagnostics GmbH; b: (Akonor et al. 2016); c: (Merdzhanova et al. 2016) and (Tabakaeva et al. 2018); d: (U.S. Department of Agriculture 2019). e: not relevant for calculation

| | Crustacean | Mollusk | Fish |
|---|---|---|---|
| **VITAL 3 RfD (organism protein, mg)** | 25 | N/A | 1.3 |
| **Conversion factor (CF) ELISA assay[a]** | 34 | 14990 | N/A |
| **Protein content of organism (%)** | 20[b] | 15[c] | 20[d] |
| **Dry weight (DW) (%)** | 25[b] | [e] | [e] |
| **Formula: assay target to organism protein** | Tpm*CF(34) *4(WW) *0.2 (prot) | Tpm*CF (14990) *0.15 (prot) | Cod eq*0.2 (prot) |
| **Factor: assay target to organism protein** | 27.2 | 2248.5 | 0.2 |

Tukey post-hoc test or unpaired t-test or Mann-Whitney test were used as appropriate and as indicated in the figure captions. Statistically different samples are shown with *$p < 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ in graphs. For graphs with superscript letters to indicate similar means (where clearer to visualize), ANOVA followed by Tukey post-hoc analysis and allocation of groups was performed in R4.3.1/RStudio. Graphs present mean values with standard deviation and individual values are shown as points.

## Results

### Variations in marine allergens between different farms, years and species

The levels of crustacean, mollusc and fish allergens in samples of the kelp species *S. latissima* and *A. esculenta* from four farms located along the Norwegian coastline, collected during the harvesting periods in 2022 and 2023, demonstrated statistically significant differences between farms, years and species as well as variation between different replicates (Fig. 2). No specific pattern of distribution was uncovered by our sampling design. The highest levels of crustacean tropomyosin were detected in *S. latissima* samples from 2023 from F.4 (the most southern of the studied farms) and were significantly higher than the corresponding samples from 2022 (that were below quantification limit), as well as significantly higher than all other samples from 2023. There were no statistically significant differences in the levels of crustacean tropomyosin between the 2022 samples of all farms. Crustacean tropomyosin was below quantification limit in all samples of F.1, which is the most northern studied farm (Fig. 2a). Mollusc tropomyosin levels were significantly higher in 2023 than 2022 for samples of *S. latissima* from F.4 and *A. esculenta* from F.1, the latter also being significantly higher than *S. latissima* samples from F.2 and F.4. in 2023. *S. latissima* samples from F.4 in 2022 had significantly lower levels of mollusc tropomyosin than *A. esculenta* samples from 2022 from F.2 and F.3 (Fig. 2b). Fish allergens were only above quantification limit in samples collected in 2022 from *S. latissima* from F.4 and *A. esculenta* from F.2, the latter having significantly higher levels of fish allergens than most other samples (Fig. 2c). Overall, for all assessed freshly collected seaweed samples from the farms (DW), the highest value for crustacean tropomyosin was 20.6 ppm (shown in Fig. 3b), the highest value for mollusc tropomyosin was 1.8 ppm (Fig. 2b) and the highest value for fish was 106.5 ppm cod equivalents (Fig. 2c).

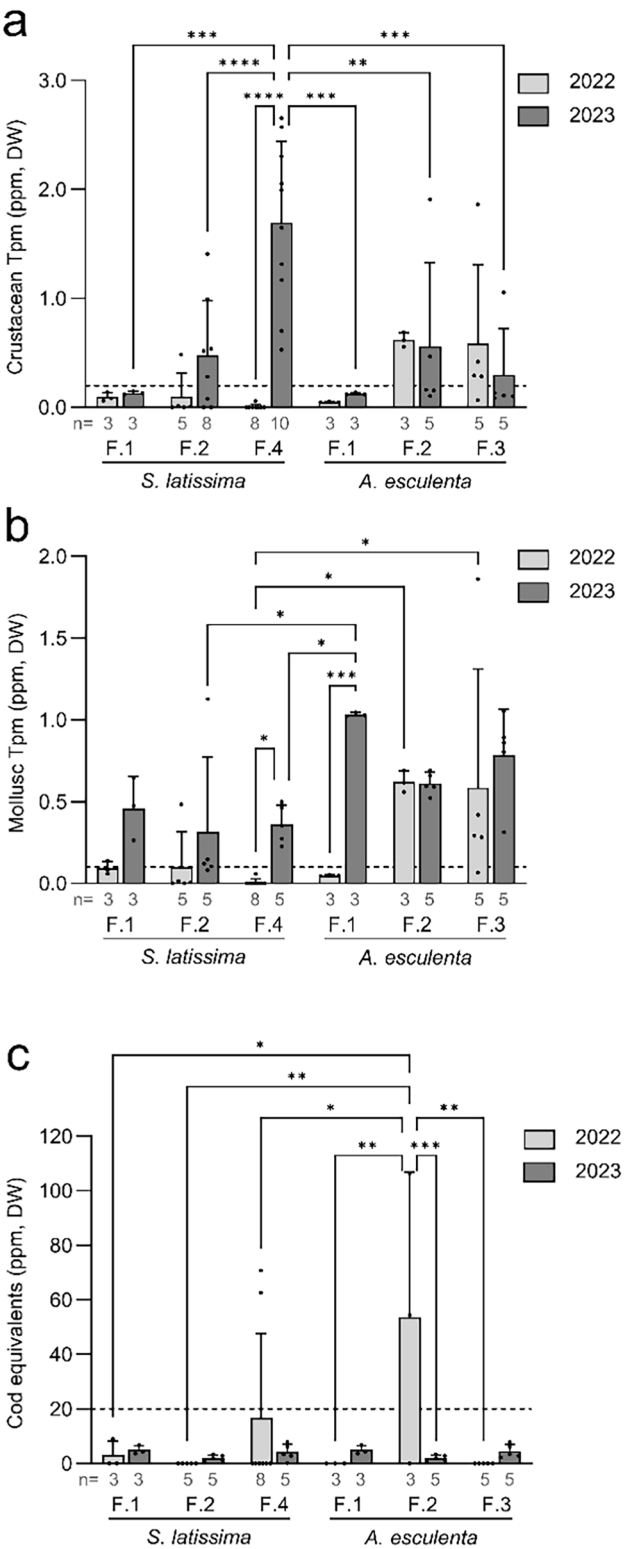


**Fig. 2** Assessment of the levels of crustacean (**a**), mollusc (**b**) and fish allergens (**c**) in samples of *S. latissima* and *A. esculenta* from Farms 1–4 (F.1-F.4), where the same species was available from 2022 and 2023. All samples were compared by mixed-effects models (a: $F_{5,21} = 10.48$, $p<0.001$; b: $F_{5,16} = 2.20$, $p=0.105$, c: $F_{5,18} = 3.13$, $p=0.033$). Shown are mean values and standard deviation as well as all single values. The number of analysed samples (n) is indicated under each bar. The dashed line indicates the lower quantification limit of the assays

### Variations in marine allergens between different locations within one farm

We also looked closer at the distribution within F.4 as one of the larger farms with both species cultured. Samples were taken from different areas inside two cultivation sites. Interestingly, crustaceans and molluscs seemed to accumulate with significantly higher levels in *A. esculenta* sampled from locations furthest downstream of the main current direction (Fig. 3b, d). This was not the case in the *S. latissima* samples (Fig. 3a, c, e) and also not for fish allergens, which were more randomly occurring (Fig. 3e, f). For crustaceans and fish, but not molluscs, differences were also seen between different samples from the same field.

### Comparison of seeding methods, macroalgae parts and harvesting timing

The seeding method of the macroalgae might influence the attraction or settlement of other marine organisms on the seaweed raft. To assess this, results from all farms are grouped together based on the seeding method used for the sample. There were significantly higher levels of crustacean tropomyosin in samples of *A. esculenta* seeded by the twine method (Supplementary Figure 1a). *A. esculenta* had significantly higher levels of mollusc tropomyosin than *S. latissima* for both direct and twine-seeded samples, with twine-seeded *A. esculenta* having the highest values (Supplementary Figure 1b). For fish allergens, no differences were seen based on the seeding method (Supplementary Figure 1c). We also compared blades and stipes of *S. latissima* harvested at F.4, but no statistically significant differences in the accumulation of marine organisms were seen (Supplementary Figure 2a-c). Harvesting one month later than usually practiced harvesting time at F.1 resulted in higher levels of crustacean and mollusc tropomyosin and fish allergenic proteins in *S. latissima*, while higher levels were measured in *A. esculenta* only for crustaceans and molluscs (Supplementary Figure 2d-f).

### Relation between observations on the farms and ELISA results

Visual observations made by the farmers were simplified to only indicate the presences or absences of fish, crustaceans and molluscs and are shown in relation to ELISA results (Supplementary Table 1). Observations and ELISA results had some corresponding trends, but observations are less detailed and more observant-dependent than the analytic results with all their variation.

### Variations in marine allergens following processing steps

Fresh macroalgae need to be processed to be stabilized and processing such as blanching has been well characterized for its potential to reduce iodine levels (Stévant et al. 2018; Jordbrekk Blikra et al. 2021; Krook et al. 2024). One aspect of this work was to assess the impact of the most commonly used processing steps on the presence of marine allergens. From one studied farm (F.3), samples of unprocessed (raw), blanched and fermented biomass of *S. latissima* and *A. esculenta* from 2022 and 2023 were analysed. Samples of *A. esculenta* from the corresponding year were also obtained from the food processor, where they had been first dried as flakes and then homogenized into powders. We did not observe a significant effect on the presence of marine allergens by blanching or fermenting, except random variation in the presence of cod in one sample (Fig. 4a-c). Drying and powdering led to some differences, however, it can be assumed that this variation arises from the mixing in bigger quantities (200 – 300 kg) as compared to the small amounts used for the experimental samples (Fig. 4d-i). Results from extracts and subsamples throughout one batch of the powdered product (Supplementary Figure 3) seemed also more stable than samples directly from F.4 (Fig. 3). An important increase in the presence of fish allergens was seen in *A. esculenta* samples after powdering, while fish allergens were not detected in the corresponding raw and flake samples (Fig. 4f).

As allergenic organisms might accumulate on certain spots in not fully homogenized samples, we further wanted to assess the risk for peak concentrations of allergens in flaked samples as compared to powdered samples. Interestingly, crustacean tropomyosin was only above quantification limit in one flake sample, while detected in all powdered samples and in both cases at a similar level (Fig. 5a). Homogenization to powder led to an increase in the mean level of crustacean tropomyosin, but also to decreased variation between samples (Fig. 5a). Mollusc tropomyosin on the other hand was decreased when assessed in powdered samples and also the variation between replicates was rather similar for flakes and powder (F-test, $p = 0.051$) (Fig. 5b). Powdered samples had higher levels of fish allergens (as already seen in Fig. 4f), while fish allergens were not detected in any of the flake samples (Fig. 5c).

### Calculation of AL transition points

To allow the risk evaluation of these ELISA results in terms of the VITAL programme, and to decide if PAL is recommended, the AL transition point can be calculated

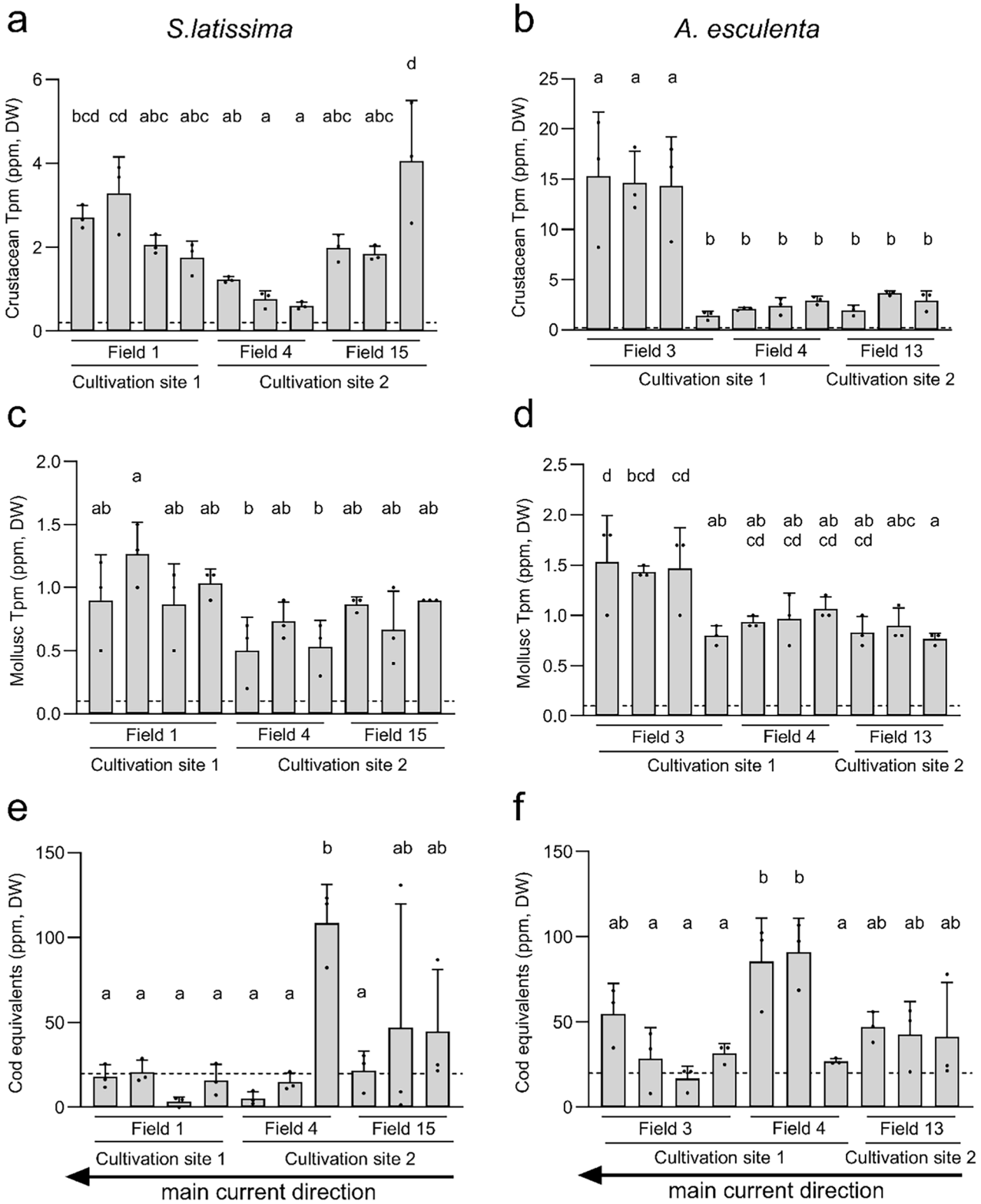


**Fig. 3** Assessment of the levels of crustacean (**a, b**), mollusc (**c, d**) and fish allergens (**e, f**) in samples of *S. latissima* (a, c, e) and *A. esculenta* (b, d, f) from farm 4 (F.4) in 2023. Three to four samples were collected from different fields (1, 3, 4, 13 and 15) of two cultivation sites (1 and 2). All samples were analysed in 3 replicates (n = 3). Samples for each species were compared by one-way ANOVA (a: $F_{9, 20} = 10.7$, $p<0.001$; b: $F_{9,20} = 13.88$, $p<0.0001$; c: $F_{9,20} = 2.93$, $p = 0.02$; d: $F_{9,20} = 4.96$, $p = 0.001$; e: $F_{9,20} = 3.86$, $p = 0.006$; f: $F_{9,20} = 5.55$, $p<0.001$). Shown are mean values and standard deviation as well as all single values. The dashed line indicates the lower quantification limit of the assay. The same superscript letters indicate statistically similar means ($p \geq 0.05$)

(Table 4). In the case of the here-discussed seaweed samples, no pre-packed food is available yet. To be on the safe side, we estimated that a RfA of 3 g dried seaweed is over the quantity of what can be expected to be ingested as one portion. All analysed samples were under the AL transition point with the suggested RfA and would not require PAL. Looking at the highest values of fish allergens in the powdered samples from the food processor with values up to 431.5 ppm cod (Fig. 5c), the total cod protein content would be 86.3 ppm and still below the AL transition point of 433 ppm fish protein. For a direct evaluation of ELISA results, AL transition points can also be expressed as amount of allergen, which is 306.4 ppm crustacean tropomyosin and 2165 ppm cod.

## Discussion

In this study, samples of cultivated kelp (*S. latissima* and *A. esculenta*) from farms along the Norwegian coastline were analysed to gain a better understanding of the levels of marine food allergens occurring in at-sea grown seaweeds. Crustacean, mollusc and fish allergens were detected in many of the kelp samples, nonetheless under the level eliciting food safety concerns. The levels of marine allergens in these samples showed differences between farms, species and years that could be due to factors not further analysed in this study such as different water temperature, salinity, pH, or surface for attachment. We observed the highest variation between and within samples for crustacean tropomyosin which was present in most samples. Mollusc tropomyosin had a more stable level across all the samples collected and fish allergens were only detected in fewer samples. Our study did not observe any specific pattern of distribution of marine allergens, what might be due to an insufficient number of samples collected at each farm to be able to document small changes in the context of high variation. Similarly, Faassen et al. (2024) have identified tropomyosin as factor with high variation, recommended to be analysed in 8 subsamples, but still giving less reliable estimates as for other food safety hazards. Overall, samples taken directly from the farms during harvesting still might indicate the allergen content for each specific harvested batch and can vary between different years, locations and species. Analysing for known marine allergens at the harvesting stage creates important information, needed for the decision making about PAL of seaweed products.

Although there was no overall effect between the different farms observed, there might be a potential for spatial variation within each single farm. Likewise, we detected differences in the occurrence of marine allergens between different locations within one studied farm. Crustacean and mollusc tropomyosin was detected at higher levels in *A. esculenta* samples from locations downstream of the main current direction, whereas not in *S. latissima* samples and not for fish allergens. If the direction of current might affect the attachment of marine organisms to the different species of seaweed would still need to be verified. We also observed differences when comparing the direct seeding method, where sporophytes are glued onto the ropes and the twine-seeding method, where sporophytes are traditionally cultured on a coil, and finally twined around the ropes (Boderskov et al. 2021). Twine-seeded samples of *A. esculenta* contained higher levels of crustacean and mollusc tropomyosin. These samples were grouped from all farms and no farm had used both methods and cultured both species for direct comparison that would be needed for causative conclusions. We further analysed the stipes of *S. latissima* that are currently not processed towards food ingredients by F.4. The stipes did not either contain levels of marine allergens that would raise food safety concerns, thus marine allergens would not be expected as a hindering factor for the development of stipe derived food products. Finally, it can be noted that the here reported levels of marine allergens in samples from farms focusing on the culture of kelp are higher than reported previously for samples from IMTA (Integrated multi-trophic aquaculture) sites (Mildenberger et al. 2021). One might expect the vicinity of other trophic species (salmon in the case of the latter study) to increase the attraction of marine organisms (James et al. 2023). Nevertheless, the findings in both studies cannot be directly compared as the samples were not collected and prepared in the same way.

We observed overall corresponding trends when comparing the observations of the farmers with the ELISA results, but not reflecting all variation of the analytic results. Still, gaining more experience with the observations at each farm and the corresponding allergenic content, might become an estimating tool for production management for the farmers themselves. Also, it is likely that observations would catch variations in the occurrence of marine allergens that would come closer to representing allergenic food safety concerns.

Certain processing methods are reported to alter allergenic proteins and eliminating allergenicity from food (Fu et al. 2019; Dong and Raghavan 2022). So, we have also tested samples that have undergone blanching and fermentation at one farm and drying and powdering at the food processor. We did not observe significant changes after blanching or fermentation by our sampling. Banach et al. (2024) analysed crustacean tropomyosin in *S. latissima* samples from F.3 in 2022 that underwent the same processing as the *A. esculenta* samples that were analysed in our study. In these samples, the highest concentration measured was 3.3 ppm crustacean tropomyosin, what is similar to the highest concentration of 3.9 ppm measured in one of the

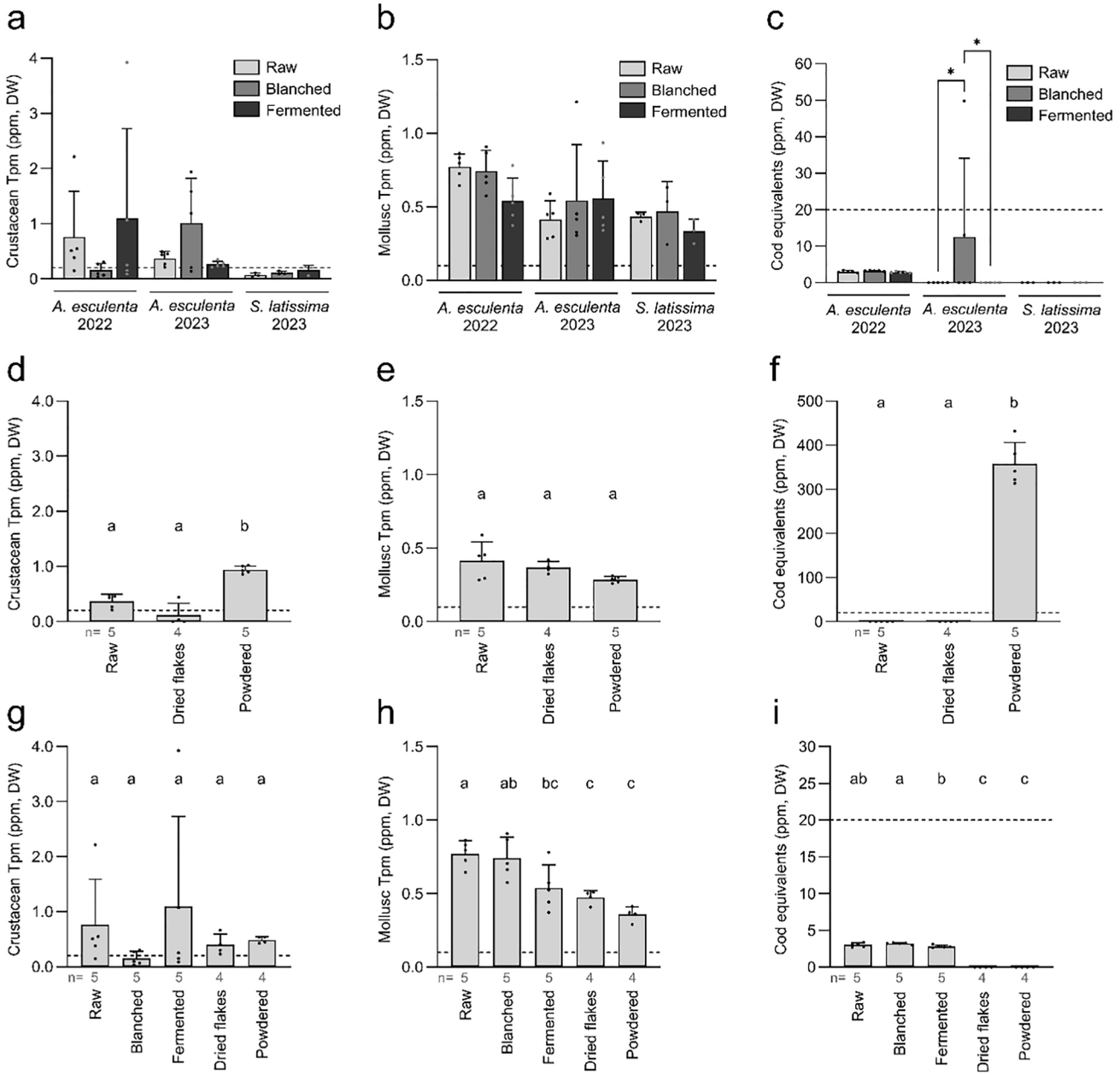


**Fig. 4** Comparison of crustacean (**a**), mollusc (**b**) and fish allergens (**c**) by two-way ANOVA for species effect (a: $F_{2,30} = 1.69$, $p = 0.201$; b: $F_{2,30} = 5.90$, $p$=0.007; c: $F_{2,30} = 0.807$, $p$=0.46) and process effect (a: $F_{2,30} = 0.070$, $p$=0.93; b: $F_{2,30} = 0.925$, p=0.41; c: $F_{2,30} = 1.208$, $p$=0.31) in unprocessed (raw), blanched and fermented samples of *A. esculenta* in 2022 and 2023 (n = 5) and *S. latissima* in 2023 (n = 3) processed by F.3 (producer). Comparison of crustacean (d), mollusc (e) and fish allergens (f) by one-way ANOVA (d: $F_{2,11} = 3.22$, p=0.08; e: $F_{2,11} = 40.8$, $p$<0.001; f: $F_{2,11} = 239$, $p$<0,001; g: $F_{4,18} = 0.85$, $p$=0.51; h: $F_{4,18} = 11.5$, $p$<0.001; i: $F_{4,18} = 440$, $p$<0.001) in samples of *A. esculenta* in 2023, raw from the producer (F.3) and dried (flakes) and powdered at the food processor. Comparison of crustacean (g), mollusc (h) and fish allergens (i) in samples of *A. esculenta* in 2022, raw, blanched and fermented from the producer (F.3) and dried (flakes) and powdered at the food processor. Shown are mean values and standard deviation as well as all single values. Single values shown for dried flakes and powder are means of 3–5 replicate extracts. The dashed line indicates the lower quantification limit of the assay

corresponding fermented samples of *A. esculenta* analysed in our study (Fig. 4a). Unlike our study, Banach et al. (2024) report an increase of the average concentration of crustacean tropomyosin after fermentation but advise to treat this result with caution due to the variability between and within batches and as only few samples had detectable levels in the preceding blanching step. Taken together, this suggests that the blanching and fermentation process at F.3 is not able to

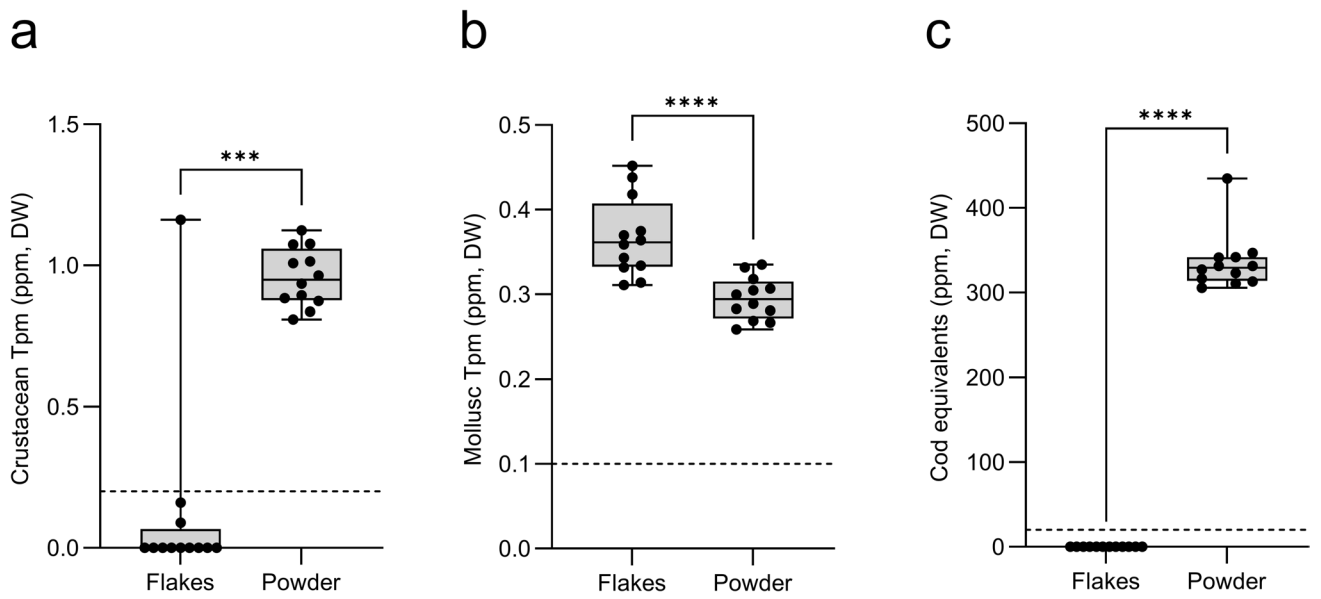


**Fig. 5** Detected levels of crustacean (**a**), mollusc (**b**) and fish allergens (**c**) from flake or powder samples from the food processor. Replicates of flake samples were separately homogenized. Due to expected differences in variances, differences were assessed by unpaired two-tailed non-parametric Mann-Whitney test (a: U=12, $n_1=n_2=12$, $p=0.0002$; b: U=8.5, $n_1=n_2==12$, $p<0.0001$; c: U=0, $n_1=n_2=12$, $p<0.0001$). Shown are min. to max. values with all single values (n = 12, 3 extracts of 4 subsamples of one batch). The dashed line indicates the lower quantification limit of the assay

reduce marine allergens in the processed seaweeds as this method may not be harsh enough to denature seafood proteins from seaweed.

When testing samples that were either only dried (flakes) or dried and powdered at the food processor, crustacean tropomyosin was only detected above quantification limit in one flake sample, whereas in all powdered samples (Fig. 5a). It is not possible to conclude if crustaceans loosen from dried flakes, while they are homogenized together with the kelp during powdering, or if crustacean tropomyosin is otherwise introduced in the powdering process. Allergens can be introduced by cross-contamination from other production lines (Allergen Bureau 2023). This was likely the case for the powdered samples tested after homogenization at the food processor and that contained the highest levels of fish allergens in this study. On the opposite no fish allergens were detected in the corresponding and preceding flake samples or samples directly collected from F.3 (Fig. 4f and i). We also observed slightly further increased levels of fish allergens throughout different subsamples of one large batch (Supplementary Figure 3c), which might be due to cross-contact from a preceding fish production. Thus, a good control and cleaning practice of production lines is at least as important as knowledge on the incoming biomass to avoid the introduction of other allergens at a later production state. Our sampling did not reveal point contamination in flake samples that was higher than in the powdered samples or that would rise food safety concerns due to marine allergens. Where seaweed biomass is intended for bulk homogenization, allergen content should be analysed after homogenization, giving values that are more stable and more relevant for documentation of the allergenic risk of the final product.

ELISA is a recognized method for the evaluation of allergens as it is based on the direct recognition of proteins (Allergen Bureau 2023). We did not observe a reduction in the levels of marine allergens that we would suspect to be due to protein denaturation, but a verification by other methods could be needed in future studies. In case of fear for false negative results due to denaturation of proteins during processing, other methods based on DNA detection might be applied for verification (Fernandes et al. 2015).

Correct allergen labelling is important to provide consumers with accurate allergen information, no matter if it is adventitious contamination or allergens as deliberate ingredients. The VITAL programme is a guideline for the food industry on how to decide if PAL is necessary for a certain product or not (Allergen Bureau 2023). We have calculated AL transition points to allow the exemplary risk evaluation of our samples in terms of the VITAL recommendations. None of the measured levels of allergenic protein in the here assessed seaweed samples were above the corresponding AL transition point for an assumed portion size of 3 g. This portion size was chosen as the double amount as reached by current inclusion rates for a single portion at the food processor (I. M. Birkeland, personal communication, 3 Oct 2023). We consider this as a realistic but conservative portion size in the light of other food safety hazards, with iodine and arsenic

currently being the most restricting factors in the tested seaweed species (Stévant et al. 2018; Krook et al. 2024). Allergen management in seaweed products is only one aspect among several possible food safety issues (Banach et al. 2024). According to the VITAL guidelines, samples below AL transition point are defined as AL1 and PAL is not recommended (Allergen Bureau 2023). Our results should serve as an example on levels of marine food allergens detected in Norwegian farmed kelp and what outcome these levels would have in terms of PAL. The results on marine food allergens in samples of macroalgae have a high variation and occurrence of marine organisms on kelp farms are prone to changes, and even more in coming years, especially as climate change is expected to impact organisms and ecosystems. Therefore, analysis should be done for all batches prior to inclusion in food products. However, the responsibility to decide if PAL is needed (or not) lies under the food producer.

## Conclusions

This study provides examples of the occurrence of marine food allergens in cultivated Norwegian kelp. Crustacean, molluscs and fish allergens were detected at varying levels in samples of *A. esculenta* and *S. latissima* collected in two consecutive years during the harvesting of the production at different farms. Focusing on different locations within one farm, crustacean and mollusc tropomyosin was detected with significantly higher levels in *A. esculenta* sampled furthest downstream of the main current direction. Higher levels of crustacean and mollusc tropomyosin were also detected in twine-seeded than in direct seeded *A. esculenta*. We did not observe significantly different content in allergens in blades and stipes analysed for *S. latissima*, but the quantity of allergens was noted to increase with an harvesting conducted at a later period than usual at one farm. On-site visual observations might be used to indicate the possible presence of allergenic organisms, but the content of allergenic proteins would need to be quantified by analyses such as ELISA. Our study did not reveal significant changes in the levels of marine allergens in samples of blanched and fermented seaweed. Homogenization in a larger batch led to less varying results, but introduced high levels of fish allergens, most likely due to cross-contamination during processing.

Overall, the kelp samples analysed in this study demonstrated a low contamination with seafood allergens and, according to VITAL recommendations, would not require PAL for an assumed portion size of 3 g dried seaweed. However, kelp cultivated at sea will always need to be examined for each batch of products as marine allergens are potentially present, might increase due to environmental variations and then, might require PAL. Further, complementary studies with a more robust number of samples for each aspect studies would be needed to document small changes of seafood allergens in products from seaweed.

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**Authors' contributions** Both authors contributed to the study's conception and design. Analyses and data curation were performed by J. M.. Conceptualization, methodology, validation, investigation, resources, writing – Original Draft, writing- review and editing, visualisation, project administration and funding acquisition for the herein presented work were conducted by both authors. Both authors commented on previous versions of the manuscript and read and approved the final manuscript.

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**Availability of data and material** All information to reproduce the presented analyses and the data supporting the findings of this study are available within the paper and its supplementary information. All numeric results are provided as Supplementary file 3.

## Declarations

**Competing interests** The authors declare no competing interests.



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