CSIRO PUBLISHING

SPECIAL ISSUE: RAAN-A 2023 | REVIEW
https://doi.org/10.1071/AN23064

ANIMAL PRODUCTION SCIENCE

Market-driven assessment of alternate aquafeed ingredients: seafood waste transformation as a case study

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Handling Editor:
David Masters

Received: 10 February 2023
Accepted: 18 April 2023
Published: 18 May 2023

Cite this:
Howieson J *et al.* (2023)
Animal Production Science, **63**(18), 1933–1948.
doi:10.1071/AN23064

ABSTRACT

With the increase in worldwide demand for seafood, the current plateau in production from wild-harvest fisheries has resulted in the rapid growth of the aquaculture sector. Aquaculture relies on quality ingredients such as fishmeal, but cost concerns have led to the investigation of a variety of alternate plant and animal by-products and microbial sources as aquafeed ingredients. Evaluation of alternative aquafeed has traditionally focused on their effects on the growth and immune status of the fish and not always on market-driven assessments of the final edible product. One of the commonly researched groups of alternative ingredients is seafood waste, which, after transformation, has potentially beneficial nutritional characteristics. Transformation, which includes rendering, enzyme hydrolysis and use as a feed source for insects and microbial species, is intended to provide stability and enhance the logistical feasibility of the waste as an aquafeed ingredient. This review discusses transformed fish waste in aquafeeds and describes some of the market and end-user implications (composition, edible safety and quality, sustainability metrics and consumer perceptions) of this approach.

Keywords: circular aquaculture, fight food waste, food processing waste, food science, full utilisation, functional additives, greenhouse gas, insect, seafood waste, single cell protein, sustainable aquaculture, upcycling.

Introduction

The contemporary drive to minimise or transform harvest and processing waste from primary production has been applied to the seafood industry (Venugopal 2021). This paper discusses the use of transformed fish-waste ingredients in aquafeeds and questions the neglect of some of the market and end-user implications (safety, quality, sustainability metrics and consumer perceptions) of including some of these ingredients.

In human nutrition, fish is an important source of protein and lipids, particularly omega-3 polyunsaturated fatty acids (PUFA), vitamins and trace elements. Regular consumption of fish at least once a week is recommended (İbrahim Haliloglu *et al.* 2004; Codabaccus *et al.* 2013; Han *et al.* 2018; Quiñones *et al.* 2021; Xu *et al.* 2021). The increase in worldwide demand for seafood, and the current plateau in production from wild-harvest fisheries have resulted in the rapid growth of the aquaculture sector. This is now estimated to provide 70% of edible seafood (FAO 2020*a*).

The aquaculture industry relies on quality aquafeed ingredients. Fishmeal (FM) produced from wild-harvested fish is still considered the best aquafeed protein source due to its favourable nutritional characteristics, excellent palatability and digestibility (FAO 2020*b*). However, the use of FM and the broadening of the gap between demand and supply has resulted in extensive investigations of protein alternatives to FM in aquafeed (Siddik *et al.* 2018*a*; FAO 2020*b*).

Plant-based raw materials have been investigated and are included in most modern commercial aquafeed (see review by Colombo *et al.* (2022)). However, the utilisation of conventional plant-based protein for finfish aquaculture, particularly for carnivorous species, faces a number of challenges. These include an imbalanced amino acid profile and antinutritional factors that can affect the growth, feed utilisation, digestibility and overall health of fish that consume them (Francis *et al.* 2001; Van Vo *et al.* 2020; Colombo *et al.* 2022). Further, together with direct competition with human food streams, utilising terrestrial crops in aquafeeds has sustainability implications, including access to freshwater, deforestation and other types of habitat modification, arboreal footprint, pesticide and fertiliser use, and nutrient run-off leading to aquatic pollution (Colombo and Turchini 2021). Various proteins derived from single-cell organisms, grown on the nutrient-rich waste streams, as next-generation protein sources in aquafeed opportunities, have also been investigated as alternate aquafeed ingredients, but are not at a viable scale of production yet (Hua *et al.* 2019).

Animal by-products (e.g. poultry by-product meal (PBM; Galkanda-Arachchige *et al.* 2020), insects (see review by Alfiko *et al.* 2022) and microbial single-cell protein (such as yeast and bacteria; see review by Jannathulla *et al.* 2021) have also been investigated to replace FM. Although these products are considered a good source of protein, their application in aquafeeds is still constrained by factors including the lack of some essential amino acids, high moisture, indigestible particles, microbial contaminants and the possibility of disease transmission (Siddik *et al.* 2019; Chaklader *et al.* 2020*a*). Aquafeed ingredients formulated from seafood waste are an alternative aquafeed opportunity and are the predominant focus of this review.

Alternate aquafeed ingredients: food and consumer science as a missing research link

Aquafeed studies using alternate ingredients have focused on technical parameters associated with the predicted growth and feed-use efficiency of the target species (such as target weight, and specific growth rate), as well as feed digestibility and palatability. Compositional considerations such as proximate, amino acid and fatty acid composition and the presence of anti-nutritional compounds have been paramount. Studies have also reported on the health and immune status of the fed fish (such as histological, blood biochemistry and gut microbiome results; Chaklader *et al.* 2021*a*; Jannathulla *et al.* 2021; Alfiko *et al.* 2022; Aragão *et al.* 2022; Chaklader *et al.* 2023*a*). Of equal importance is the consideration of operational/supply parameters for the inclusion of novel ingredients, such as their consistency of quality, cost, transport logistics and scale of production or supply (Hua *et al.* 2019). More recently, sustainability assessments such as greenhouse-gas (GHG) emissions and circularity have also become a focus (Colombo *et al.* 2022).

The interdisciplinary approach of measuring edible food safety and the food quality of the aquaculture product (in addition to the above parameters) has often been neglected when experimenting with alternate aquafeed formulations (Chaklader 2021). This oversight is in contrast to other animal production industries where the analyses of feed types in relation to the final edible meat safety and quality are more advanced (Costa *et al.* 2021).

This lack of focus on food science is surprising, as not only does food science research address the fundamental purpose of aquaculture (to ensure stable and reliable sources of food supply) by evaluating the safety of end products, but it also assists in maintaining or improving the quality of farmed fish, to ensure that products remain competitive in the market (Floros *et al.* 2010; Calanche *et al.* 2020; Tacon *et al.* 2020).

Food science and aquaculture

Compositional analyses of food allow for precise nutritional labelling and can lead to putative health claims. It is therefore noteworthy that the composition of seafood species grown with alternate ingredients is often provided, and has been shown to be affected by diet (Olsen *et al.* 2004; Mai *et al.* 2006; Moren *et al.* 2006; Olsen *et al.* 2006; El-Rahman and Badrawy 2007; Abdul Kader *et al.* 2011; Kader and Koshio 2012; Friesen *et al.* 2013; Gause and Trushenski 2013; Waagbø *et al.* 2013; Sprague *et al.* 2015; Emery *et al.* 2016; Wong *et al.* 2016; Kim *et al.* 2019; Monge-Ortiz *et al.* 2020; Chaklader *et al.* 2022, 2023*b*). This alteration of composition, particularly evident in fatty acid content, has consequences for human nutrition (Blondeau *et al.* 2015; Mensink 2016; Michielsen *et al.* 2019). Further, this compositional variation will affect a range of food science perspectives, including nutritional messaging and labelling, food safety, quality, and shelf-life (Table 1). Thus, the broader picture of compositional variation should be considered when evaluating novel aquafeed ingredients (Hixson 2014). Lastly, when undertaken, compositional analysis is often completed on the whole fish (including viscera, skin and head) rather than on the edible portion (e.g. fillet), necessitating a secondary analysis relevant to the latter.

Safe seafood and quality seafood

In terms of food science, the production of safe food is of primary importance, as it directly affects consumer health (Tritscher *et al.* 2013; Wong *et al.* 2016). Food safety can be divided into two components, namely, chemical and microbiological safety. In aquacultured seafood, the microbiological risk is mostly introduced post-harvest (during processing). Therefore, for alternate aquafeed investigations, the chemical safety of the final aquaculture products, specifically heavy metals and persistent organic pollutants (Fernandes *et al.* 2018; Sheng and Wang 2021), is the main focus. Levels of these compounds in the edible products are variously regulated in seafood safety legislation.

In addition to food safety, the quality of seafood is a complex topic and covers a wide range of end user-driven definitions, including nutritional, microbiological, sensory and physicochemical characteristics (Nielsen *et al.* 2002). In the field of food science, the quality of fish is often analysed as its perceived ‘eating quality’ and ‘freshness’, which can

Table 1. Summary of existing studies on impacts of non-plant-based fishmeal replacement on heavy metal contamination in fish.

Fish species	Alternative feed ingredients (FM replacement %)	Analysed portion	Analysed heavy metal	Outcomes	References
Red sea bream (*Pagrus major*)	Fermented soybean and scallop by-product (0–60%)	Whole fish	Cd, Pb	[A]Significant increase in Cd and Pb in fish after fed for 45 days, with Cd significantly higher in the diet and Pb being insignificantly different from test diet	Abdul Kader *et al.* (2011)
Red sea bream (*P. major*)	Mixture of fish solubles, fermented soybean and squid by-product (0–100%)	Fillet	Cd, Pb	Significant increase in Cd in diet (0.58 mg/kg vs 2.34 mg/kg); no Cd or Pb was detected in final fillet	Kader and Koshio (2012)
Atlantic cod (*Gadus morhua*)	Antartic krill (*Euphausia superba*; 0–100%)	Fillet	As, Cd, Hg, Pb	Higher Cd concentration (0.61 mg/kg vs 0.19 mg/kg) in test diet than in control diet; no significant differences in final fillets.	Moren *et al.* (2006)
Atlantic salmon (*Salmo salar*)	Artic krill (*Thysanoessa inermis*) or amphipod (*Themisto libellula*; 0–40%)	Fillet	As, Cd, Hg, Pb	Higher Cd (1.4 mg/kg, 12 mg/kg vs 0.19 mg/kg) and Pb (0.22 mg/kg, 0.16 mg/kg vs 0.09 mg/kg) concentration in artic krill and amphipod respectively, than in test diet; no significant differences were found in final fillet	Moren *et al.* (2006)
Japanese seabass (*Lateolabrax japanicus*)	Squid viscera (6–16%)	Fillet and internal organs	Cd	Up to 27.5 (0.21 mg/kg vs 12.08 mg/kg) times higher in Cd concentration when comparing test diet with control, but no Cd was detected in muscle tissue. Significant increases were found in gill, liver, and kidney	Mai *et al.* (2006)
Nile tilapia (*Procambarus clarkia*)	Crayfish (*Procambarus clarkia*), crayfish by-product (0–50%) or treated crayfish (0–100%)	Fillet	Cd, Pb	[A]Significant increase in Pb and Cd on fish muscle when fed with 50% crayfish by-product meal (1.60 mg/kg vs 0.40 mg/kg; 0.206 vs 0.141 mg/kg respectively).	El-Rahman and Badrawy (2007)
Nile tilapia (*P. clarkia*)	Tuna by-product (40–70%)	Fillet, internal organs, and whole body	Cd, Hg	[A]Significant increase in Hg in muscle portion when comparing 40–70% replacement diet regardless of fish weight. Increase in muscle weight also increased concentration of Hg in fillet. Cd content increased significantly in muscle when fish was over 500 g. Accumulation of Hg and Cd in internal organs increased significantly in 70% replacement, regardless of fish weight.	Kim *et al.* (2019)
Mediterranean yellowtail (*Seriola dumerili*)	Mixture of corn gluten, krill, meat and bone (33–66%)	Fillet	As, Cd, Hg	Significant reduction of As content compared with FM diet (1.4 mg/kg vs 0.81 mg/kg); insignificant differences in Cd and Hg.	Monge-Ortiz *et al.* (2020)

[A]Significant increases in heavy metal accumulation were identified in final product.

influence end-user demand, and hence competitiveness of products on the market (Nielsen *et al.* 2002).

The conventional techniques to determine the quality of seafood can be divided into two types, quantitative human sensory evaluation and instrumental analytical determination. The human sensory analyses include appearance, colour, texture, odour and taste (Nielsen *et al.* 2002; Wu *et al.* 2019). The analytical attributes include pH, total volatile base-nitrogen (TVB-N) (protein deterioration) and thiobarbituric acid-reactive substances (TBARS, lipid oxidation; Olafsdóttir *et al.* 1997; Aro *et al.* 2003; Grigorakis *et al.* 2004; Olafsdottir *et al.* 2004; Iglesias *et al.* 2009; Yao *et al.* 2011; Itoh *et al.* 2013; Cheng *et al.* 2015; Chung *et al.* 2021; Chaklader *et al.* 2022, 2023*b*). When sensory and instrumental analytes and acceptable limits are combined with microbiological assessment, then the shelf-life of the target products can be estimated. All these quality analyses will affect the consumer experience, from purchase to consumption (Nielsen *et al.* 2002; Wu *et al.* 2019).

Consumer science in relation to the perceptions and acceptance of new aquafeed ingredients on purchasing behaviour should also be considered, understood and addressed as part of any multi-disciplinary approach to new aquafeed ingredient investigations (Chaklader 2021; Colombo *et al.* 2022). It is paramount to ensure that human sensory quality is the same or better than for traditionally cultured fish, as appearance is one of the characteristics used for decision-making by the purchaser (Colombo *et al.* 2022).

Fish waste and the possibilities for transforming it into aquafeed

Transformed fish-processing waste is now being considered as an alternate aquafeed ingredient and can partially replace FM, or alternatively be a supplement-fed in conjunction with other plant-, animal-, or microbial-based ingredients. Feeds developed from transformed fish waste have the advantage of delivering essential nutrients (e.g. amino acid and fatty acid, vitamins, macro-minerals and trace elements) that may be lacking in other (non-FM) alternate formulations, as well as improving palatability (Colombo et al. 2022). Depending on inclusion rates, such fish-based ingredients should produce an edible food product with compositional characteristics aligned to those produced from FM (Colombo et al. 2022). Incorporation of fish waste into aquafeeds would also better meet the sustainable development goals aligned to reduced food loss, and thus increase the opportunity for circularity in the aquaculture industry (Colombo et al. 2022; UN 2023; Fig. 1).

In Australia, it is estimated that between 50 000 (Arcadis 2019) and 100 000 t of waste (Dundas-Smith and Huggan 2006) are produced by seafood industries every year, costing an estimated AUD15 million per annum for disposal (He et al. 2013). An estimated 50 000 t per annum is generated at the manufacturing stage (from whole fish to fillets; Arcadis 2019), as approximately 60% of the fish is discarded during filleting (Chalamaiah et al. 2012). Waste, includes skin, heads, muscle, viscera, liver, and bones. Internationally, it is estimated that ~40% of the total seafood supply is wasted among harvesting, production and processing (Love et al. 2015; Laso et al. 2016) and, in Europe, it is estimated that seafood losses and wastage rates are greater than 30% (FAO 2012). In consideration of fish waste at the harvest stage, the terms 'bycatch' and 'discards' are applied where the undersized, low-valued or unintentional catch are caught or discarded while fishing for target species. While regulations are not yet implemented in Australia, the EU 'Landing Obligation (Common Fishery Policy (Regulation EU No. 1380/2013)' (European Union 2013) now prevents discard from the vessel, and hence profitable use of the bycatch is under greater consideration (Colombo et al. 2022).

Aside from aquafeed ingredients, fish and seafood wastes have been widely researched for a variety of outcomes, including fertiliser, pet food, edible food, nutraceuticals and supplement products (Venugopal 2021; Nag et al. 2022). The main limitation to the effective use of fish waste is predominantly perishability, resulting in reduced product quality and

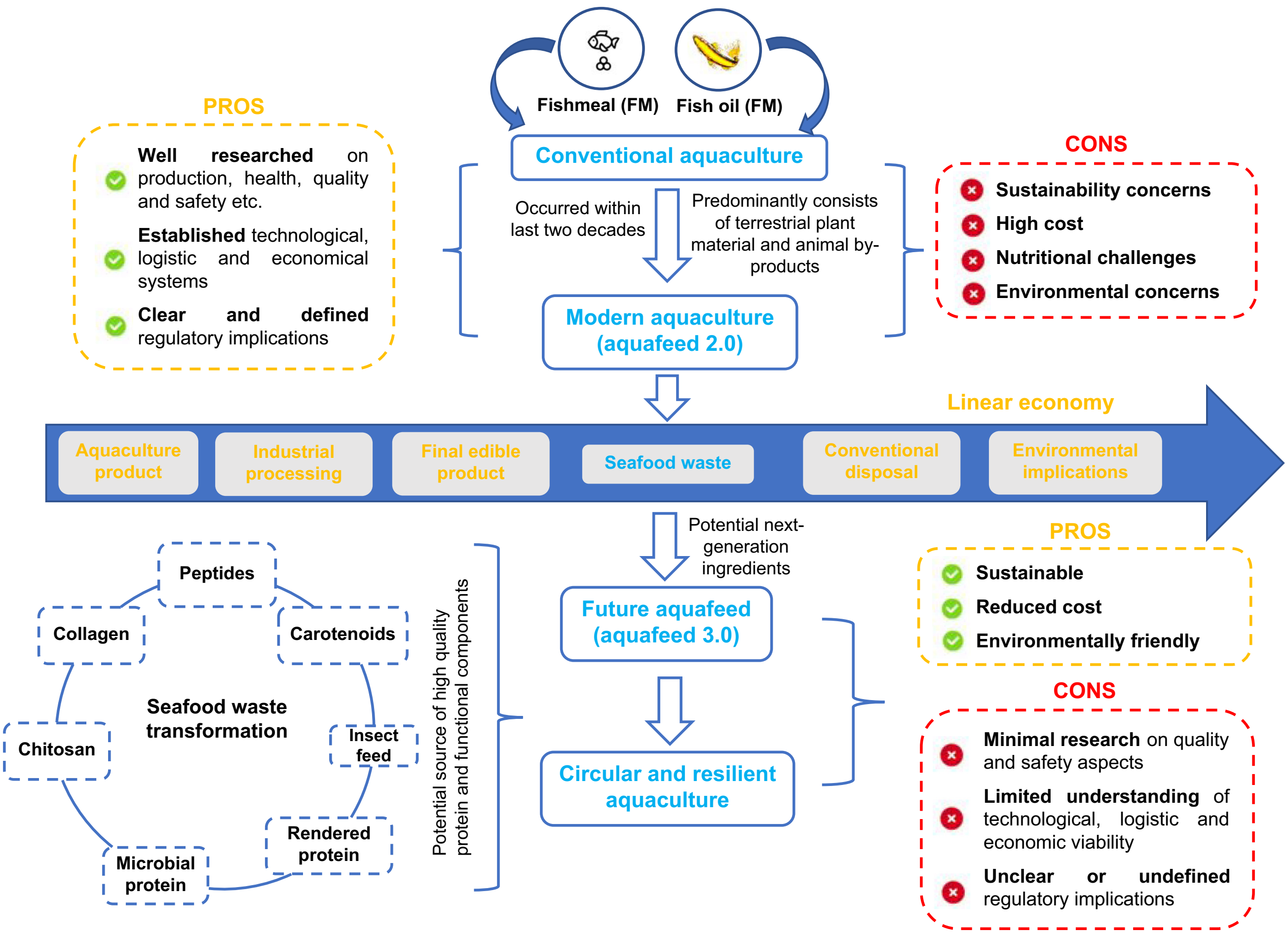

Fig. 1. Schematic representation of seafood-waste transformation into various next-generation alternative protein sources and sustainable additives for future aquafeed (aquafeed 3.0). This is proposed to overcome several feed constraints generated by conventional aquaculture and modern aquaculture (aquafeed 2.0). The idea was adapted from the study of Colombo et al. (2022) and Venugopal (2022).

consistency. This is partly due to the disparate locations of the low volumes of fish waste, affecting the ability to reach an economic scale sufficient for processing and transportation. The lack of suitable-scale infrastructure has also been identified as a barrier (Hua *et al.* 2019). Hence, while there is some work on the addition of untreated fish waste to small-scale aquaculture operations (Bechtel 2007), transforming the perishable raw seafood material into high-quality stable products, and their transport logistics, has been the focus of recent research efforts (Arulkumar *et al.* 2018; Lerfall *et al.* 2018; Olatunde and Benjakul 2018*a*, 2018*b*; Lambrianidi *et al.* 2019; Zhaleh *et al.* 2019; Baptista *et al.* 2020; Castro *et al.* 2020; Shokri *et al.* 2020; Ucar *et al.* 2020; Hoque *et al.* 2022).

This article will focus on three types of transformational processes for fish waste, and opportunities for the extraction of specific supplemental compounds. We will, first, briefly consider the use of these ingredients in aquaculture nutrition, with reference to the growth and immune status of the fed fish. Where data are available, we will discuss the impact of the alternate feed ingredients on food safety and the quality of the final edible product. Sustainability and circularity considerations will follow as well as a discussion of limitations and barriers to widespread commercial implementation.

Fish-waste transformation processes for aquafeed ingredients

Rendering of fish-processing waste. Rendering refers to the drying of seafood by-products. Rendered by-product usually has lower protein and higher ash concentration than does high-quality FM derived from whole fish, which contains 66–74% crude protein, 8–11% crude lipids, and <12% ash (Hua *et al.* 2019). In comparison, white fishmeal produced from rendered by-products contains 60–67% crude protein, 7–11% crude lipids, and 21–23% ash, and tuna fishmeal produced from rendered by-products contains 57–60% crude protein, 8–14% fat, and 12–21% ash (Goddard *et al.* 2008; Hernández *et al.* 2014; Jeon *et al.* 2014; Kim *et al.* 2014; Ween *et al.* 2017; Hua *et al.* 2019). The lower protein and higher ash content in by-product FM are not unexpected, as the nutrient composition differs among whole fish, fillets, and other parts of the body (viscera, heads, skin, bones, and blood). The different proportions of variously rendered by-products that are added to fishmeal will therefore also contribute to the nutrient variability of the feed (Hua *et al.* 2019)

Nonetheless, rendered by-products are currently added to aquafeed as a partial replacement for FM, without apparent compromise. For example, growth was maintained at replacement rates of 15.8–21.4% in spotted rose snapper (*Lutjanus guttatus*), and up to 30% in olive flounder (*Paralichthys olivaceus*) at a dietary inclusion rate of 21%. For Korean rockfish (*Sebastes schlegeli*), 75% of FM could be substituted by tuna by-product meal at a dietary inclusion rate of 58.1%, without compromising growth and feed utilisation (see summary by Hua *et al.* (2019)).

Enzyme hydrolysis of fish waste. The process of hydrolysis converts fish waste to fish protein hydrolysate (FPH) in liquid or dried form. There are several hydrolysis methods including chemical hydrolysis (acid and alkaline hydrolysis), autolysis, bacterial fermentation and enzymatic hydrolysis (Siddik *et al.* 2021). Enzymatic hydrolysis is widely implemented to produce precise hydrolysates that retain the nutritive value of the source protein (Zamora-Sillero *et al.* 2018). Enzyme hydrolysis targets specific peptide bonds and amino acids. It produces a consistent-quality product, while excluding any residual organic solvents or toxic chemicals in the end-products (Najafian and Babji 2012). Product stability in storage and transport can also be optimised (Siddik *et al.* 2021).

An extensive review by Siddik *et al.* (2021) summarised enzyme hydrolysis from fish-processing waste in a wide range of seafood species and by-product raw materials, including skin, heads, muscle, viscera, liver and bones. FPH products were reported to be a good source of protein, peptides, and amino acids. The process of hydrolysis results in the breakdown of larger protein molecules into smaller, more bioactive compounds, and hence FPH has been reported to possess desirable functional and bioactive peptides. A moderate inclusion of FPH in aquafeeds has the potential to improve growth, feed utilisation, immune response and disease resistance of a wide range of fish (see review by Siddik *et al.* (2021)).

Our laboratory conducted several studies to convert Australian seafood-production waste, including tuna (*Thunnus maccoyii*) and kingfish (*Seriola lalandi*), to FPH, so as to assess their subsequent utilisation in aquafeed. We reported that the replacement of FM with >20% tuna hydrolysates negatively affected the welfare of barramundi (*Lates calcarifer*). The hydrolysates were rich in free amino acids, and reported to act as anti-nutritional factors (Siddik *et al.* 2018*b*). However, a 56-day feeding study that supplemented 5–20% of tuna hydrolysates against a reference diet (FM-based) indicated that 5% and 10% supplementation improved the growth of barramundi, improved haematology indicators, gut mucosal barrier function, immune response and disease resistance against *Streptococcus iniae* (Siddik *et al.* 2018*a*). The beneficial effects of tuna hydrolysates shown by this study motivated us to supplement a low-quality protein ingredient (poultry by-product meal) with 5% and 10% tuna and kingfish hydrolysates (Chaklader *et al.* 2020*b*). We found that these hydrolysates when added to poultry-by product meal could completely replace FM, with a significant improvement in feed utilisation, growth, mucosal barrier function and immunity in barramundi, compared with an FM-only diet. Importantly, supplementation with 10% tuna hydrolysate enhanced the gut microbial diversity, along with a positive influence on beneficial bacteria (Siddik *et al.* 2020).

Similarly, several protein hydrolysates from seafood waste have recently been tested on other aquaculture species (Chaklader *et al.* 2020*b*). One study found that supplementation of plant protein with 10% whole blue whiting (*Micromesistius poutassou*) hydrolysate allowed higher inclusion of plant protein into the diet of Atlantic salmon (*Salmo salar*) and increased the growth rate relative to controls (Egerton *et al.* 2020). The authors also found an improved essential amino acid bioavailability in the blood of the same dietary groups. In a further study, a fishmeal-based diet top-coated with 2% tuna hydrolysate improved the digestibility, immune responses and disease resistance of red sea bream (*Pagrus major*) and olive flounder (*P. olivaceus*) against *Edwardsiella tarda* (Khosravi *et al.* 2015).

Insects: black soldier fly larvae fed on fish waste. Although a number of different insect species have been investigated for aquafeed ingredients (see review by Alfiko *et al.* (2022), black soldier fly (*Hermetia Illuscens*) larvae (BSFL) is considered the most promising owing to its amino acid composition (Hua *et al.* 2019)). This insect species possesses the capacity to valorise low-value organic waste or by-products into highly nutritious biomass while requiring less arable land and water consumption, as well as reduced CO_2 production (Henry *et al.* 2015). The final biomass contains high protein and lipid but this may vary depending on the substrate and processing method (Wang *et al.* 2019).

A number of studies have investigated BSFL in aquafeed across a range of fish species and inclusion rates. However, in most of these studies, the growth on BSFL substrates was not well articulated. The main disadvantage of BSFL incorporated directly into aquafeed is the presence of high amounts of saturated fatty acids and a negligible amount of some essential polyunsaturated fatty acids (Alfiko *et al.* 2022). In our work, we initially demonstrated that the fatty acid profile of the BSFL could be manipulated using fish waste from carp as the growth substrate (Tilley *et al.* 2019). In our following studies, BSFL meal grown on 70% carp waste and 30% agriculture waste was mixed with poultry by-product meal (PBM). Results indicated that adding 10–30% BSFL meal to PBM could replace FM completely in the barramundi diet (Chaklader *et al.* 2019; Chaklader *et al.* 2020*c*; Chaklader *et al.* 2021*a*, 2021*b*). We reported that by feeding BSFL on fish waste the lipid profile of the ensuing larval meal was optimised for aquafeed (Tilley *et al.* 2019). Some defatting further improved the value of BSFL meal in aquafeed formulations.

Microbial valorisation of seafood waste. There are several single-celled organisms, including marine protists such as *Aurantiochytrium*, *Schizochytrium* and *Thraustochytrium*; methanotrophic bacteria such as *Methylobacterium* and *Methylococcus*; chemotrophic proteobacteria such as *Clostridium* and *Baccillus*; yeasts such as *Candida*, *Cyberlindnera*, *Kluyveromyces*, *Rhodotorula*, *Saccharomyces* and *Wickerhamomyces* that have been tested for use as aquafeed single-cell ingredients (Colombo *et al.* 2022). Many of these studies have identified the potential to cultivate these microorganisms in seafood- and aquaculture-processing wastes. Even though the industry is still in its infancy, 20 major producers of microalgae and cyanobacteria, and 16 major producers of protists, yeasts and bacteria have already been identified by a recent industry report (Krishfield *et al.* 2019). A recent review comprehensively illustrated the potential of using single-cell ingredients as next-generation sources of protein and/or lipid in fish and shrimp aquafeeds (Colombo *et al.* 2022).

Microalgae such as *Chlorella* spp., *Spirulina* spp., *Dunaliella* spp., diatoms, and cyanobacteria, are promising agents for the bioconversion of seafood waste. Their digestive actions allow the degradation of organic contents, unused food, and excretory products, together with the removal of CO_2, NH_3-N, CO_2, and H_2S, thereby ameliorating environmental pollution (Puyol *et al.* 2017; Gifuni *et al.* 2019). The algal biomass contains high protein (60%) and oil (75% with high n-3 PUFA contents) and is also a good source of polysaccharides, minerals, and pigments including chlorophylls, carotenoids, and phycobiliproteins (Stengel and Connan 2015; Venugopal 2021). These nutrient and functional molecules have motivated the use of microalgae as a source of bioactive peptides, animal feeds, food additives, and as probiotics in aquaculture, as illustrated in the reviews of Venugopal (2021). Further, several aerobic, anaerobic, or facultative microbes could be used to detach food components by various microbial fermentation techniques including solid-state, liquid-state, or submerged-state fermentation, environmentally friendly, safe, and cost-effective techniques (Nag *et al.* 2022). Small food components derived from fermentation or fermented product could be used as functional ingredients in aquafeed formulation (see the review of Nag *et al.* (2022)).

Fish waste-based aquafeed ingredients: seafood safety and quality considerations

Seafood safety. Chemical contaminants that can accumulate in fish via feed include a wide range of persistent toxic substances (PTS) such as inorganic heavy metals, and persistent organic pollutants (POPs) (Karl *et al.* 2003; Lundebye *et al.* 2004; Tritscher *et al.* 2013; Hixson 2014; Wong *et al.* 2016). In fish fed with alternative non-plant-based ingredients, the majority of the published research has focused on four main elements, namely, cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) (Table 1).

In a summary of studies involving the use of seafood by-products originating from either scallops, crayfish or tuna as a FM replacement, approximately 38% identified heavy metal contamination in the final fish products (El-Rahman and Badrawy 2007; Abdul Kader *et al.* 2011; Kim *et al.* 2019). Drilling down further into these data showed that accumulation was related to the portions analysed and the fish species. In one of the heavy metal studies that identified a significant increase in the Cd and Pb content, the authors suggested that the results were most likely to be due to the inclusion of waste internal organs in the analysis (Abdul Kader *et al.* 2011). It is well known that heavy metal accumulation from feed mostly occurs in the viscera of the fish, especially in the liver and kidney, while in comparison, the muscle portion of the fish is unaffected (Berntssen *et al.* 2000; Mai *et al.* 2006; Abdul Kader *et al.* 2011; Paschoalini and Bazzoli 2021). In the study conducted by Mai *et al.* (2006), despite a 27.5 times higher Cd concentration in the test diet than in the control (12.08 mg/kg, 0.21 mg/kg respectively), no Cd increase was detected in the fillet.

However, in another study, Nile tilapia (*Oreochromis niloticus*) accumulated heavy metals in the fillets. It is uncertain what caused this anomaly, since various factors including biological and water parameters could all affect the susceptibility of fish towards metal accumulation (Jezierska and Witeska 2006; Ali and Khan 2018; Paschoalini and Bazzoli 2021).

Seafood quality

A feature of the academic literatures on aquaculture feed studies, where rendered by-products and FPH have been examined as an alternate ingredient, is the paucity of information on the food safety and quality of the edible product. However, we do note that a broader examination of various animal by-products included in feed (e.g. PBM; blood and bone meal) indicated no detrimental impacts on final fillet quality (Williams *et al.* 2003; Chaklader *et al.* 2021*c*) (Table 2).

In a promising outcome, BSFL meal was shown to improve final fillet quality in rainbow trout (*Oncorhynchus mykiss*; Borgogno *et al.* 2017) and barramundi (*L. calcarifer*; Chaklader *et al.* 2022, 2023*b*). In these three studies, an improvement in flavour and texture, especially juiciness, was reported. As well, a reduction in colour intensity was observed in fish fed with BSFL (Belghit *et al.* 2019; Chaklader *et al.* 2022, 2023*b*). While a lighter colour is preferred in white-fleshed fish such as barramundi, it might not be preferable in coloured-flesh fish such as salmon (Alfnes *et al.* 2006; Chaklader *et al.* 2022, 2023*b*). This study also showed improved sensory quality and lipid oxidation in the BSFL-fed fillets in an 8-day shelf-life experiment. It is noteworthy that in the studies by Chaklader *et al.* (2022, 2023*b*), BSFL was produced partially on fish waste; however, the food source was not clear in other studies.

These reports underpin the acceptance of seafood waste as a substrate to enrich PUFA in BSFL (one of the limiting factors in BSFL meal in aquadiets). In the contradictory results reported by Belghit *et al.* (2019), the inclusion of BSFL caused a significant increase in undesirable flavours. This may have been due to the growth substrate (which was not clearly articulated). Further studies are needed to produce BSFL by using seafood waste and other food waste as substrates, with their subsequent utilisation in aquafeed in a commercially relevant trial to confirm the potential of BSFL to improve the final product quality in aquaculture production.

Can supplementary ingredients from fish waste extend the shelf-life of fillets? Functional ingredients extracted from fish waste and added to aquafeed can result in an extension to shelf-life (Fig. 1). A recent review examined the potential of FPHs as edible coatings to preserve food (Tkaczewska 2020). Protein hydrolysates embedded into edible packaging effectively inhibited pathogenic microorganisms and lipid oxidation in fish products (Tkaczewska 2020). As noted previously, there is a paucity of data on the shelf-life and quality of aquacultured fillets produced with FPH supplementation. It might also be possible that FPH addition to aquafeed results in the assimilation of bioactive peptides not only to enhance growth performance and immune status in the target species but also as a means to improve product quality.

Carotenoids such as astaxanthin (commonly used in salmon aquaculture) can be obtained from the discards of crab, salmon, and prawn processing (Nag *et al.* 2022). Carotenoids are biomacromolecules possessing antioxidant potential associated with lipid peroxidation (Nag *et al.* 2022). The contemporary assimilation of carotenoids via an aquaculture diet may also play a role in elevating the shelf-life of aquaculture products (Nag *et al.* 2022). Similarly, chitin is present in discarded components of seafood and may be partially deacetylated by enzymatic hydrolysis to chitosan (Pati *et al.* 2020; Pati *et al.* 2021). In alkaline conditions, or under the influence of a chitin deacetylase, it exhibits antimicrobial, antioxidant, and antiviral properties (Li *et al.* 2010). This has motivated researchers to examine chitosan as a coating to extend the shelf-life of fish products (and other animal products), as detailed in the review of Kumar *et al.* (2020) and Socaciu *et al.* (2018). Chitosan supplementation as an antioxidant, growth promoter and immunostimulant in aquatic animals has been reviewed by Abdel-Ghany and Salem (2020); however, the effect on aquaculture product quality is unknown.

Emissions, resource use and circularity considerations of transformed fish waste as aquafeed ingredients

Sustainability: GHG emissions, water and energy use. Contemporary markets also assess sustainability credentials. In assessing GHG emissions associated with fish waste use in aquaculture, there is a separation required between

(a) the GHG emissions associated with current outcomes for waste production in the seafood supply chain, and
(b) the GHG emissions associated with the conversion of seafood waste to a specific aquafeed ingredient.

There is also a growing impetus to understand the GHG emission variation for the final edible product, including the impact of different feed formulations. This last separation is not covered in this review as it has been the subject of multiple other reviews and reports (Hua 2021; Ruiz-Salmón *et al.* 2021; Blueshift Consulting 2022; Ziegler *et al.* 2022).

In the context of sustainability within seafood supply chains, there are some advantages to utilising fish waste (Murali *et al.* 2021). GHG emissions (carbon dioxide equivalent (CO_2 eq) from two Western Australian finfish supply chains,

Table 2. Summary of existing studies on impacts of non-plant-based feed ingredient replacement on physicochemical attributes in fish.

Fish species	Alternative feed ingredients (FM replacement %)	Type of methods	Investigated parameters	Outcomes	References
Mediterranean yellowtail (*S. dumerili*)	Mixture of corn gluten, krill, meat and bone (33–66%)	Analytical and sensory evaluation	Analytical: colour, moisture, pH, texture	[A]Significant changes in colour, especially increase brightness [A]Significant reduction in adhesiveness, chewiness, gumminess, and hardness	Monge-Ortiz *et al.* (2020)
			Sensory: appearance, odour, colour, texture taste	[A]Significant in marine aroma, whiteness and water retention were detected by sensory panel	
Japanese seabass (*Lateolabrax japonicus*)	Mixture of poultry by-product meal, meat and bone meal, spray-dried blood meal and hydrolysed feather meal in 40:35:20:5 ratio (20–80%)	Analytical and sensory evaluation	Analytical: texture	[A]Significant increase in hardness, chewiness, cohesiveness, and reduction in adhesiveness on fish fed with test diet	Hu *et al.* (2013)
			Sensory: appearance, odour, colour, texture, taste, overall preference	[A]Consumer significantly favour fish fed with control diet	
Barramundi (*Lates calcarifer*)	Mixture of poultry by-product meal and black soldier fly larvae (*Hermetia illucens*) (100% in 85:15, 80:20, 75:25)	Analytical and sensory evaluation	Analytical: drip loss, colour, texture, structural changes, Ph, lipid oxidation	[A]Significant increase in brightness and yellowness in fish fed with test diet [A]Test diet suppressed rancidity of fish during storage	Chaklader *et al.* (2022)
			Sensory: quality index evaluation, appearance, odour, texture, taste, overall preference	[A]Consumer significantly prefered fish fed with test diets	
Barramundi (*L. calcarifer*)	Mixture of poultry by-product meal and defatted or full-fat black soldier full larva (*Hermetia illucens*) (100% in 70:30)	Analytical and sensory evaluation	Analytical: drip loss, colour, texture, structural changes, Ph, lipid oxidation	[A]Defatted diet improved the texture profile of fish [A]Significant increase in brightness in fish fed with test diet [A]Increase fish resistance to degradation during storage	Chaklader *et al.* (2023*a*)
			Sensory: quality index evaluation, appearance, odour, texture, taste, overall preference	[A]Consumer significantly prefer fish fed with test diets [A]Increase fish resistance to degradation during storage	
Atlantic salmon (*S. salar*)	Black soldier fly larvae (25–100%)	Sensory evaluation	Sensory: taste, texture	No significant differences were identified	Lock *et al.* (2016)
Atlantic salmon (*S. salar*)	Black soldier fly larvae (0–100%)	Sensory evaluation	Sensory: appearance, odour, colour, texture, taste	[A]Significant increase in rancid odour and off-odour of fish fed with test diet [A]Significant reduction in colour intensity of cooked salmon in fish fed with test diet [A]Significant softer in raw salmon, significant harder in cooked salmon in fish fed with test diet	Belghit *et al.* (2019)
Rainbow Trout (*O. mykiss*)	Black soldier fly larvae (25–50%)	Sensory evaluation	Sensory: differences test	No significant differences were identified	Sealey *et al.* (2011)
Rainbow Trout (*O. mykiss*)	Mixture of poultry by-product, blood and feather meal (0–100% in 27:5:5, 25:3:3)	Sensory evaluation	Sensory: preference, odour, taste, texture	[A]Significant increase in grassy flavour and softness of fish [A]Significant reduction in fish aroma	Craft *et al.* (2016)
Rainbow Trout (*O. mykiss*)	Black soldier fly larvae (25–50%)	Analytical and sensory evaluation	Analytical: texture	No differences in shear force	Borgogno *et al.* (2017)
			Sensory: description, colour, odour, texture, taste	[A]Significant reduction in overall aroma and increase in overall flavour [A]Significant improvement in texture, including juiciness and tenderness	

(Continued on next page)

Table 2. (*Continued*).

Fish species	Alternative feed ingredients (FM replacement %)	Type of methods	Investigated parameters	Outcomes	References
Gilthead sea bream (*Sparus aurata*)	Blood or haemoglobin meal (0–10%)	Analytical and sensory evaluation	Analytical: colour Sensory: differences test	No differences in colour [A]Significant differences were detected in all test diets beside 5% blood meal	Martínez-Llorens *et al.* (2008)
Pacú (*Piaractus mesopotamicus*)	Bovine plasma protein concentrate (0–100%)	Analytical and sensory evaluation	Analytical: texture, colour Sensory: colour, odour, taste, texture	[A]Significant increase in hardness and chewiness [A]Significant increase oily mouthfeel, flavour intensity, foreign flavour, firmness and chewiness	Pavón *et al.* (2018)
Hybrid striped seabass (white bass *Morone chrysops* × striped bass *M. saxatilis*)	Poultry by-product meal (~6%)	Analytical and sensory evaluation	Analytical: colour Sensory: differences test	No differences in colour No differences compare with control diet	Turek *et al.* (2020)
Atlantic salmon (*S. salar*)	Poultry by-product oil (80%) or mixture of 1:1 tallow and poultry by-product oil (80%)	Sensory evaluation	Sensory: preference, taste	No differences between both diets	Mai *et al.* (2006)
Brown trout (*Salmo trutta* L.)	Poultry fat or pork lard (0–100%)	Sensory evaluation	Sensory: odour, colour, texture, taste	No differences between both diets	Turchini *et al.* (2003)

[A]Significant changes in physicochemical attributes were identified in final products.

from harvest to retail outlet, were assessed using streamlined life-cycle methodology and cleaner production strategies (Denham *et al.* 2016). Electricity consumption contributed to the highest GHG emissions within the supply chains, followed by leakage of refrigeration gas and landfill disposal of unused fish portions (calculated as 62.5% wastage by weight). By isolating this waste during processing and developing compost using the methods of López-Mosquera *et al.* (2011), a reduction in the GHG of 5.8% and 1.2% was measured from the regional and city supply chains respectively.

A recent audit of GHG emissions in the Australian fishing and aquaculture sectors found that 45% of the emission profile was derived from feed/bait, transport, and processing-related costs (termed Scope 3 emissions), followed by 31% from Scope 1 emissions (fuel and fugitive emissions from refrigerant gases etc.; Blueshift Consulting 2022). The study did not specifically address the effect of interventions to reduce or re-use processing waste but did consider the impact of the aligned landfill disposal of these materials.

Efficient utilisation of wastes and by-products could also decrease the volume of waste generation and correspondingly the energy and water consumed by their treatment (Tomczak-Wandzel *et al.* 2015). Kurniasih *et al.* (2018) reported that the minimisation and conversion of waste generated in the seafood industry could potentially result in a saving of 27.2% of clean water.

There has been little work on GHG emissions from novel aquafeed ingredients generated on fish waste, in the context of their transformation to aquafeed components. However, feed manufacturers, in particular the larger companies such as Skretting and BioMar, are now taking significant steps in advanced carbon accounting methodologies for the raw materials used in their feeds (Blueshift Consulting 2022). Maiolo *et al.* (2020) reported that only a small number of studies dealt with an evaluation of feed components on an individual basis, including alternative ingredients for salmon aquafeed formulations (Pelletier and Tyedmers 2007); FM and fish oil production in Peru (Fréon *et al.* 2017); several aquafeed ingredients commonly used in Indonesia (Henriksson *et al.* 2017); and alternative meal and fat (or oil) sources (Silva *et al.* 2018). In this study, Maiolo *et al.* (2020) found that insect meal had a similar impact to PBM on GHG emissions, and was more efficient than were microalgae and macroalgae as potential ingredients.

Circularity. Developing next-generation feed supplements for aquafeed ingredients via a circular economy (Fig. 1) that does not deplete natural resources, and may have positive impacts to reduce the environmental footprint, is the opportunity awaiting future sustainable and resilient aquaculture (Chaklader *et al.* 2021*a*). Food loss and waste associated with the seafood industry, and the knock-on effect of triggering an increased fishing effort to meet increasing market demand, perpetuates the linear economy in seafood production (Ruiz-Salmón *et al.* 2021). Aquafeed ingredient formulation from fish waste is, therefore, an opportunity to close the loop in the seafood industry, through the valorisation of waste streams, and subsequent utilisation in other industries, while eventually being fed back to the original industry (de la Caba *et al.* 2019).

Limitations and barriers to fish-waste transformation for aquafeed ingredients

The limitations to the future use of seafood waste in the development of alternate aquafeed ingredients, particularly in an Australian context, are described below.

Technical, logistical and economic factors. Worldwide, there are logistical barriers to accumulating the minimum quantities required for fish-waste transformation to be economically viable (Hua *et al.* 2019). In Australia, local processing is often of insufficient volume for cost efficiency, multiple species are harvested and Australian labour costs are high (Cunningham *et al.* 2022). Large volumes of waste are needed for viable fish-waste transformation, and the transport of perishable material from disparate locations results in quality and consistency issues in the final product (Cunningham *et al.* 2022). High electricity costs are also a barrier to storing sufficient products to increase scale. Upfront capital costs, and investment costs without appropriate incentives, make alterations to fish-waste utilisation in these production systems difficult, especially for small businesses (Cunningham *et al.* 2022). Nonetheless, there are some examples of such transformation, in particular the enzyme hydrolysis of tuna waste in Port Lincoln and its subsequent application in horticulture and aquaculture (Howieson *et al.* 2017).

In regard to waste transformation via insects, while it is estimated that insect-meal production was ~10 000 t in Australia in 2020, and will rise to 0.5 million t by 2030, these volumes are still much lower than the present production levels of protein feeds and co-products (~5 million t of FM used/year; Alfiko *et al.* 2022). This industry will require significant investment, research, and development to mature into a viable, competitive commodity. To minimise the existing high production costs, more research into automation processes is required. While production remains low, the price will continue to be a barrier to the wider adoption of insect proteins generally, and in aquaculture specifically (Alfiko *et al.* 2022).

Political, legal and regulatory factors. Despite regulatory changes relating to animal by-products and insect transformations, biosecurity concerns remain a barrier to reprocessing organic waste and circularity. For example, proteins from the waste of one species cannot be used to feed the same species, to avoid cannibalism, but can be fed to other species. There are also other biosecurity barriers; feed for salmon in Australia is not allowed to include waste from other fish industries such as tuna from Thailand (Cunningham *et al.* 2022). The regionality of raw materials and transport to a central hub is also problematic as different States and Territories have different transport and border regulations.

Since insect species are able to convert biowastes into protein sources, sanitation measures for the safe use of substrate must be developed to ensure that insect meals are free of diseases and undesirable elements. The use of insect meals as a replacement for FM in aquafeeds requires the ongoing development of legal frameworks and legislation, as well as the improvement of risk-assessment procedures (Alfiko *et al.* 2022).

End-use: safety, quality, and consumer perception considerations

Food safety. The need to examine edible portions of aquacultured fish for heavy metal contamination resulting from aquafeed ingredients grown on viscera has been previously explained (Table 1). Another food-safety issue to be considered is the persistent organic pollutants (POPs), which include a wide range of compounds such as dioxins, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Berntssen *et al.* 2000; Hixson 2014; Sprague *et al.* 2015). In fact, the consumption of fish has been regarded as one of the major dietary exposure routes for POPs (Sprague *et al.* 2015). The risk of bioaccumulation of heavy metal and POPs is a barrier to commercialisation of novel feed ingredients. Hence, where the viscera of fish might be valorised, the safety of the final edible product should not be overlooked.

The gap in knowledge of the impact of alternate rendered by-products and FPH on edible product quality and shelf-life has been discussed. Similarly, it is also necessary to research the impact of feeding aquaculture species with different sources such as insect meals on the safety, quality, and societal acceptance of those seafood (Alfiko *et al.* 2022). Positive consumer perception of by-products may increase their viability (Hua *et al.* 2019).

Future considerations, opportunities and directions

We contend that end-user/market-driven considerations of the final edible product when trialling new aquafeed ingredients are often neglected. Specifically, to fully investigate the opportunities for use of fish waste in aquafeed ingredients, and to better assess some of the market/end-user implications (safety, quality, sustainability and perceptions), the following areas of market-driven investigation are recommended:

(a) A better understanding of the impact of different aquafeed ingredients on food science characteristics, including compositional analysis of the edible component, food safety, sensory and biochemical assessment, when compared with traditional feed formulations, and shelf-life.
(b) A better understanding of the importance of consumer perception of aquacultured products fed on alternate ingredients.
(c) A better understanding of the sustainability assessments of individual aquafeed ingredients used in feed, and the impact of reduction of food waste in the seafood supply-chain life-cycle.

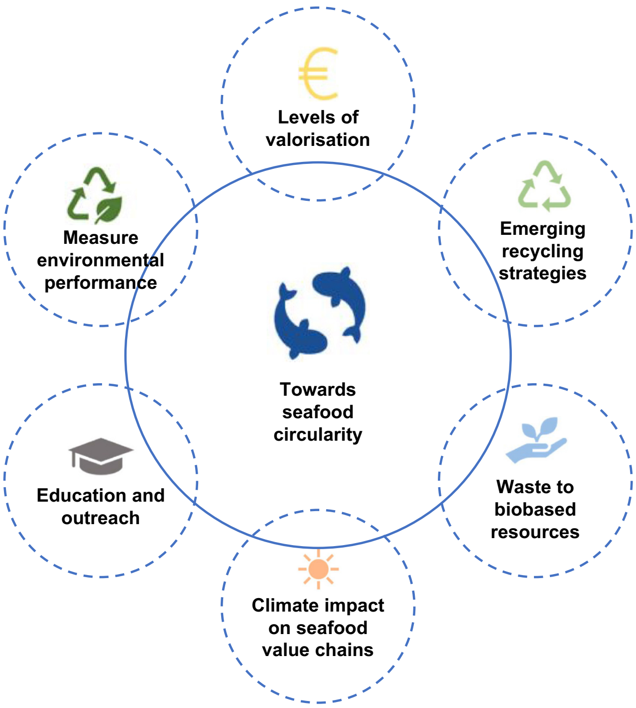

Fig. 2. The potential areas that need to be implemented for increased uptake of seafood circularity in seafood value chains, adapted from the study of Cooney *et al.* (2023).

(d) Economic and logistical feasibility assessments and initiatives to overcome commercialisation barriers associated with scale, transport and infrastructure (Fig. 2).

(e) A number of multidisciplinary and holistic stages, as illustrated in Fig. 2, will need to be developed and implemented to increase circularity in the seafood value chains.

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Data availability. The authors support transparency and reproducibility of research, to access information used in this review, email reaz.chaklader@dpird.wa.gov.au.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. This study was supported by the Research Training Program (RTP) Stipend Scholarship, funded by Australian Government to Md Reaz Chaklader (No. 19061054-Curtin), FRDC 2016.180 (Assessment of options for Virus affected carp) and FRDC 2013/711.40 (New options for Seafood Processing Waste Seafood).

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