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Review

Ulva spp. as a natural source of phenylalanine and tryptophan to be used as anxiolytics in fish farming

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ABSTRACT

Species of the green macroalgae genus *Ulva* often exhibit rapid growth, are generally cosmopolitan, and are rich in amino acids, vitamins, proteins, and minerals and have high potential for commercial uses. *Ulva* aquaculture was established and experimentally integrated into fish and shrimp farming in Brazil as Integrated Multi-Trophic Aquaculture projects. Decreases in fish farm production are often due to deaths caused by stress – with consequent increases in production costs. Essential amino acids, such as tryptophan and phenylalanine, have been used in fish farms as anxiolytic agents. In that context, a bibliographic survey was carried out to investigate advances during the last 17 years in the use of tryptophan and phenylalanine produced by *Ulva* species in fish farming. The biosynthesis patterns of tryptophan and phenylalanine were also examined in the research data. References to the presence of tryptophan and phenylalanine in *Ulva* spp. were encountered in 32 articles, with *Ulva lactuca* being the species most cited. References to the use of essential amino acids as anxiolytics in fish farming were encountered in 23 articles, with tryptophan being the most cited; none of the articles, however, mentioned the use of *Ulva* spp. as sources of anxiolytics. Temperature and pH were the factors that most influenced phenylalanine production. In conclusion, there is a potential role for the use of selected species of *Ulva* in fish farming as sources of tryptophan and phenylalanine for anxiolytic purposes.

1. Introduction

As human populations increase, concerns related to food security have also increased. Aquaculture is viewed as one of the best means to meet those needs (Godfray et al., 2010; FAO, 2018). In 2016, mariculture and coastal aquaculture produced 28.7 million tons (US$67.4 billion) of food (FAO, 2018). The mortality of farmed fish is frequently caused by stress related to management activities such as transport, biometric determinations, pathological analyses, hormonal implants, and reproduction induction (Barton, 2000; Hseu et al., 2003; Martins et al., 2013; Wolkers et al., 2014; Zaminhan et al., 2017). To minimize that problem, essential amino acids (EAA) such as phenylalanine (Phe) and tryptophan (Trp) have been examined for their anxiolytic activities (Herrero et al., 2007; Li et al., 2009; Martins et al., 2013; Zaminhan et al., 2017).

As Phe and Trp and other EAA are found in the green seaweed *Ulva*, the species of that genus could improve fish health (Madibana et al., 2017) and production, although the results will vary according to the *Ulva* species used and local abiotic factors (Angell et al., 2015).

In order to determine the potential value of *Ulva* as an anxiolytic in fish farming, as well as the abiotic factors that can improve their EAA production, we describe in this review the current state of knowledge (as presented in published scientific articles) of the use of Trp and Phe in fish farming, the presence of those EAA in *Ulva* spp., their protein contents, as well as the Phe synthesis patterns of *Ulva* spp. according to their geographic locations (latitude and longitude), considering environmental data obtained from the BioOracle data set.

A bibliographic survey was carried out using the following data bases: Periodical of CAPES/MEC (2000-2018), Web of Science (2000-2018), Science direct (2000-2018), Scifinder (2000-2018), and Google Scholar (2000-2018). Articles from January 2000 to April 2018 were searched using the key-words: amino acids *Ulva* fish-farming; amino

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acids *Enteromorpha* fish-farming; phenylalanine *Ulva*; phenylalanine *Enteromorpha*; tryptophan *Ulva*; tryptophan *Enteromorpha*; phenylalanine fish-farming; tryptophan fish-farming; phenylalanine pisciculture; and tryptophan pisciculture. The results obtained from the literature are presented here in tables containing the following information: the *Ulva* species; the research aim; the respective concentrations of Phe and Trp. To further detail the use of EAA in fish farming, the articles were classified according to the fish species and family, fish developmental phase, Phe and Trp concentrations, and the respective aims of each study. The names assigned to the alga taxa were checked in the AlgaeBase (Guiry and Guiry, 2019); the fish names were confirmed using FishBase (Froese and Pauly, 2019).

2. Effects of stress on fish production

Stress in fish farming can be related to physical (e.g., handling, transport, confinement, or capture), chemical (e.g., pollutant exposure, acidification, low oxygen, temperature), or biotic stressors (e.g., startling, the presence of predators) that can affect physiological responses such as growth, reproduction, immune system functioning can trigger fish mortality (Barton, 2002; Wolkers et al., 2012, 2014). In accordance with the magnitude and duration of the stressor, the fish can be impacted at molecular to biochemical levels (Barton, 2002). Additionally, the degree and the type of behavioral and physiological responses to stress can vary among fish species (El-Khaldi, 2010).

According to Barton (2002), three physiological responses are usually attributed to environmental stressors that require physiological adjustments in terms of metabolism, respiration, etc. The primary responses include initial neuroendocrine alterations, the release of catecholamine from chromaffin tissue, and the stimulation of the hypothalamic-pituitary-interrenal axis – resulting in the release of corticosteroid hormones. Secondary responses are responsible for variations in plasma and tissue ion and metabolite levels, hematological features, and heat-shock or stress proteins. Tertiary responses are related to animal performances (e.g., changes in growth rates, disease resistance, behavioral patterns, and survival).

Since fish management triggers fish mortality and production loss, the search for new sources of anxiolytics from ecofriendly sources is of great importance.

3. Phenylalanine and tryptophan as anxiolytics in fish farming

Phe can be converted to tyrosine, which is a precursor of dopamine and the neuro-transmitters responsible for stress responses in fish (Li et al., 2009; Saavedra et al., 2009). Trp is a precursor of serotonin (5-Hydroxytryptamine, 5-HT), a neurotransmitter responsible for the regulation of behavioral processes and growth and protein synthesis (Hseu et al., 2003; Höglund et al., 2005; Pewitt et al., 2017).

Of the 25 articles that reported the use of Phe and Trp as anxiolytics in fish farming, 24 cited the use of Trp; only one cited Phe (Table 1). Preference should be given to Trp, as it is a serotonin precursor that can minimize fish stress caused by factors such as handling and cannibalism (Wolkers et al., 2012, 2014). Trp is also a precursor of niacin (Vitamin B3), which stimulates insulin production – an important growth hormone (Table 1) – resulting in improvements in the zoo-technical parameters of the fish.

Supplementing Trp in the diet of *Rhamdia quelen* (Quoy and Gaimard, 1824) increased its growth and improved feeding efficiency. When Trp concentrations were lowered, feeding efficiency decreased (Pianesso et al., 2015) – showing that it is important to animal growth (related to the production of insulin). Similarly, the inclusion of Trp in the diets of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) and *Cyprinus carpio* (Linnaeus, 1758) resulted in greater growth uniformity (Tang et al., 2013; Zaminhan et al., 2017) – a very important consideration in commercial animal production.

The EAA Trp occurs in low concentrations in nature, making investigations focusing on it (and other sources of EAA) necessary and commercially important (Li et al., 2009; Le Floc'h et al., 2011).When absent in fish diets, or administered at only low concentrations, their growth diminishes – demonstrating the existence of species-specific requirements (Pianesso et al., 2015; Pewitt et al., 2017). Supplements of Trp in the diet of *Oncorhynchus mykiss* (Walbaum, 1792), for example, did not decrease their aggressive behavior or their growth (Winberg et al., 2001; Lepage et al., 2002), but it did diminish cannibalism in *Sander lucioperca* (Linnaeus 1758) (Król and Zakęś, 2016). An inadequate dose of Trp was probably used in the former case, which resulted in an imbalance of amino acid ratios, again demonstrating the dose-specific effect of that compound.

Phe is an EAA also used as an anxiolytic in fish farming, being critical to metabolic regulation, growth, and stress responses in the form of the neuro-transmitter dopamine (Li et al., 2009; Saavedra et al., 2010; Zehra and Khan, 2014). *Solea senegalensis* (Kaup, 1858) fed with supplemental Phe showed significant stress reductions – a response attributed to the greater induced synthesis of dopamine (Costa et al., 2012). As such, the literature reviews verified that the use of Phe and Trp in fish farming can be very important to fish health.

4. Phenylalanine and tryptophan in *Ulva* spp.

Thirty-two scientific articles with information concerning Phe and Trp in *Ulva* spp. were encountered. Twenty-two had used similar analytical methods for detecting EAA in *Ulva* spp. The quantification of EAA in *Ulva* spp. in most of the studies was related to their nutraceutical properties and, to a lesser extent, bio-refining. None of the articles discussed the anxiolytic potentials of the EAA, Phe, or Trp found in *Ulva* spp. (Table 2). *U. lactuca* (39%), *U. fasciata* (10%), and *U. rigida* (10%) were the most-cited macroalgae.

Phe concentrations in *Ulva* species ranged from 0.001–36.7 g per 100 g of protein; Trp concentrations ranged from 0.6–0.7 g per 100 g of protein. The range of Phe values reflect different extraction protocols, analytical instrumentation, as well as spatial and temporal variations of the alga sampled, among other factors (Kumar et al., 2017). Of the 32 articles analyzed, only one quantified Trp; Phe was quantified in all of them (Table 2). That result is related to the use of acid hydrolysis for extracting EAA – which makes it impossible to detect Trp (Lourenço et al., 2002; Yaich et al., 2011; Angell et al., 2016, 2017); the article that quantified Trp used an alkaline hydrolysis extraction process (Bikker et al., 2016). In the 31 articles that did not detect Trp, the probable intentions of the authors were to determine total amino acid contents – leading us to advise that searches for anxiolytic substances in *Ulva* should employ alkaline hydrolysis.

5. *Ulva* spp. as candidates for IMTA

Among the different seaweeds, species of the green macroalgae *Ulva* are candidates for use in aquaculture (Silva et al., 2015; Shpigel et al., 2017) as sources of amino acids, proteins, minerals, fibers, and other constituents (Valente et al., 2006; Ergün et al., 2009; El-Tawil, 2010; Angell et al., 2015; Moustafa and Eladel, 2016). Additionally, *Ulva* species have ideal characteristics for aquaculture due to their rapid growth and wide tolerance of abiotic conditions (Castelar et al., 2014), including their use as biological filters within the integrated multi-trophic aquaculture model – IMTA, as they show considerable rates of nutrient assimilation (Neori, 2008; Cruz-Suárez et al., 2010). That group of algae also shows promise as a co-feed in IMTA with shrimp (Pallaoro et al., 2016), as a protein supplement for gilt-head bream fish, and as a biofilter to reduce nitrogen levels (Shpigel et al., 2017, 2018). With the increased demand for protein biomasses as feed ingredients, improvements of *Ulva* spp. cultivation could provide another source of amino acids; *Saccharina latissima* kelp, for example, cultivated in IMTA, produces abundant amino acids (Marinho et al., 2015).

Ulva spp. can also be used as ingredients in animal feed due to their

Table 1

Articles with quantification (in g.kg^{-1}) and types of use of tryptophan (Trp) and phenylalanine (Phe) in fish farming between 2000 and 2017.

Taxa/Taxon used in article/Common global name	Family	Development Phase	Amino acid	Quantification (g.kg^{-1})	Response	Author/year
Cirrhinus mrigala (Hamilton, 1822) Mrigal carp	Cyprinidae	Juvenile	Trp	9.4	Reduction of aggressive behavior	Wolkers et al., 2012
		Juvenile	Trp	9.4	Stress reduction	Wolkers et al., 2014
Cirrhinus mrigala (Hamilton, 1822) Mrigal carp	Cyprinidae	Juvenile	Trp	1.0	Increased growth	Ahmed and Khan, 2004
		Juvenile	Trp	1.4	Stress reduction and increased growth	Tejpal et al., 2009
Gibelion catla (Hamilton, 1822) *Catla catla* (Hamilton, 1822) Catla	Cyprinidae	Juvenile	Phe	16.9	Increased growth	Zehra and Khan, 2014
Cyprinus carpio (Linnaeus, 1758) Carp	Cyprinidae	Juvenile	Trp	0.1	Increased osmotic tolerance	Hoseini and Hosseini, 2010
		Juvenile	Trp	3.1	Increased tolerance to copper toxicity	Hoseini et al., 2012
		Juvenile	Trp	3.8	Increased growth	Tang et al., 2013
Dicentrarchus labrax (Linnaeus, 1758) European seabass	Moronidae	Juvenile	Trp	0.5–2.5	Ineffective	Papoutsoglou et al., 2005
		Juvenile	Trp	0.5	Stress reduction	Herrero et al., 2007
		Juvenile	Trp	0.5	No anti-inflammatory activity	Machado et al., 2015
Diplodus sargus (Linnaeus, 1758) White seabream	Sparidae	Larvae	Trp	4.0	Reduction on growth	Saavedra et al., 2010
Epinephelus coioides (Hamilton, 1822) Orange-spotted grouper	Serranidae	Juvenile	Trp	3.6	Reduction of aggressive behavior, cannibalism	Hseu et al., 2003
Heteropneustes fossilis (Bloch, 1794) Stinging catfish	Heteropneustidae	Juvenile	Trp	0.1	Increased growth	Ahmed, 2014
Morone chrysops (Rafinesque, 1820) X *Morone saxatilis* (Walbaum, 1792) White bass/ Striped bass	Moronidae	Juvenile	Trp	2.1	Increased growth	Gaylord et al., 2005
Morone chrysops (Rafinesque, 1820) X *Morone saxatilis* (Walbaum, 1792) White bass/ Striped bass	Moronidae	Juvenile	Trp	2.5	Death	Gaylord et al., 2005
Oncorhynchus mykiss (Walbaum, 1792) Rainbow trout	Salmonidae	Juvenile	Trp	0.2	Stress reduction	Winberg et al., 2001
		Juvenile	Trp	0.5–3.6	Stress reduction	Lepage et al., 2002
Oreochromis niloticus (Linnaeus, 1758) Nile tilapia	Cichlidae	Juvenile	Trp	4.5	Stress reduction	Martins et al., 2013
		Juvenile	Trp	2.9	Increased growth	Zaminhan et al., 2017
Rhamdia quelen (Quoy & Gaimard, 1824) South American catfish	Heptapteriadae	Juvenile	Trp	2.5–3.4	Increased growth	Pianesso et al., 2015
Salmo trutta (Linnaeus, 1758) Sea trout	Salmoniadae	Juvenile	Trp	3.0	Attenuate anorexia stress	Hoğlund et al., 2007
Sander lucioperca (Linnaeus, 1758) Pike-perch	Percidae	Larvae	Trp	5.1–20.0	Stress reduction	Król and Zakęś, 2016
Sciaenops ocellatus (Linnaeus, 1766) Red drum	Scianidae	Juvenile	Trp	2.8	Increased growth	Pewitt et al., 2017

Table 2
Articles with quantification of phenylalanine (Phe) and tryptophan (Trp) in g of amino acid per 100 g^{-1} protein, of various *Ulva* spp. between the years 2000 and 2018 (Author/year) in studies on the chemical composition (CC), nutritional composition (NC) and feed (F).

Taxa/as *Enteromorpha* in the article	Phe	Trp	Study	Author/year
U. californica Wille	6.3	–	NC	Pirian et al., 2016
	6.3	–	NC	Pirian et al., 2016
U. capensis Areschoug	4.0	–	F	Shuuluka et al., 2013
U. clathrata (Roth) C. Agardh	3.8	–	F	Cruz-Suárez et al., 2009
	3.7	0.7	CC	Peña-Rodríguez et al., 2011
	3.7	0.6	CC	Peña-Rodríguez et al., 2011
	4.2	0.7	CC	Peña-Rodríguez et al., 2011
	4.4	0.6	CC	Peña-Rodríguez et al., 2011
U. compressa Linnaeus	0.8	–	NC	Paiva et al., 2017
	8.9	–	NC	Pirian et al., 2016
	8.8	–	NC	Pirian et al., 2016
= *E. compressa*	5.6	–	NC	Ganesan et al., 2014
= *E. compressa*		–	CC	Chattopadhyay et al., 2007
U. fasciata Delile	0.5	–	CC	Lourenço et al., 2002
	0.5	–	CC	Lourenço et al., 2002
	5.7	–	NC	Moustafa and Eladel, 2016
	6.2	–	NC	Moustafa and Eladel, 2016
	5.7	–	NC	Moustafa and Eladel, 2016
	6.1	–	NC	Moustafa and Eladel, 2016
	6.2	–	NC	Moustafa and Eladel, 2016
	6.0	–	NC	Moustafa and Eladel, 2016
	5.5	–	NC	Moustafa and Eladel, 2016
	5.9	–	NC	Moustafa and Eladel, 2016
	5.5	–	NC	Rameshkumar et al., 2013
	6.7	–	NC	Pirian et al., 2016
	6.8	–	NC	Pirian et al., 2016
U. flexuosa Wulfen	9.2	–	NC	Pirian et al., 2016
	9.1	–	NC	Pirian et al., 2016
= *E. tubulosa*	5.8	–	NC	Ganesan et al., 2014
U. intestinalis Linnaeus	> 0.0	–	NC	Benjama and Masniyom, 2011
	0.3	–	NC	Ramos et al., 2000
= *E. intestinalis*	4.9	-	CC	Biancarosa et al., 2017
	7.4	-	NC	Maehre et al., 2014
U. lactuca Linnaeus	5.4	0.7	NC	Bikker et al., 2016
	1.3	–	F	Ortiz et al., 2006
	0.8	–	F	Pallaoro et al., 2016
	4.0	–	F	Shuuluka et al., 2013
	> 0.1	–	NC	van der Wal et al., 2013
	5.7	–	CC	Wong and Cheung, 2001
	2.5	–	F	Yaich et al., 2015
	3.7	–	NC	Yildirim et al., 2009
	0.6	–	NC	Kumar and Kaladharan, 2007
	4.9	–	NC	Nielsen et al., 2012
	4.2	–	NC	Rodríguez-González et al., 2014
	6.0	–	NC	Maehre et al., 2014
	9.5	–	NC	Pirian et al., 2016
	9.6	–	NC	Pirian et al., 2016
	4.3	–	CC	Astorga-España et al., 2016
	5.6	–	CC	Biancarosa et al., 2017
U. linza Linnaeus	9.2	–	NC	Pirian et al., 2016
	9.3	–	NC	Pirian et al., 2016
= *E. linza*	5.7	–	NC	Ganesan et al., 2014
= *E. linza*	3.6		NC	Yildirim et al., 2009
U. ohnoi M. Hiraoka and S. Shimada	6.0	–	CC	Angell et al., 2015
	6.2	–	CC	Angell et al., 2015
	8.5	–	CC	Mata et al., 2016
U. pertusa Kjellman	< 0.1	–	NC	Benjama and Masniyom, 2011
U. prolifera O. F. Müller	7.9	–	NC	Pirian et al., 2016
	7.8	–	NC	Pirian et al., 2016
U. reticulata Forsskal	5.3	–	NC	Ratana-arporn and Chirapart, 2006
	0.3	–	CC	Al-Saif et al., 2014
U. rigida C. Agardh	3.3	–	F	Shuuluka et al., 2013
	4.8	–	CC	Taboada et al., 2010
	17.8	–	NC	Paiva et al., 2017
	1.2	–	CC	Gao et al., 2017
	1.1	–	CC	Gao et al., 2017
U. tepida Masakiyo and Shimada	9.6	–	NC	Carl et al., 2016
	11.1	–	NC	Carl et al., 2016

desirable nutritional characteristics, often acting as nutraceuticals (Shuuluka et al., 2013; Shpigel et al., 2017; Wells et al., 2017). Additionally, the use of *Ulva* spp. cultivated under IMTA conditions as feed could replace up to 10% of the fishmeal otherwise provided (Abdel-Warith et al., 2016), diminishing production costs (purchase, transport, and storage) while increasing profits and better ensuring the sustainability of fish farming.

The demand for alternatives to the use of animal proteins in human diets has grown considerably due to awareness of cardiovascular diseases and diabetes linked to high levels of saturated fats and cholesterol (Bleakley and Hayes, 2017). *Ulva* spp. are rich in proteins and amino acids such as alanine, arginine, phenylalanine, leucine, proline, threonine, tryptophan, and valine (Li et al., 2009; Shuuluka et al., 2013; Angell et al., 2015; Mata et al., 2016; Pallaoro et al., 2016), and the inclusion of *Ulva* spp. as a food supplement in shrimp and fish farming has improved animal health (Cruz-Suárez et al., 2009; Shuuluka et al., 2013; Pallaoro et al., 2016). Since the cultivation of *Ulva* species has been found to be viable in integrated aquaculture systems (Castelar et al., 2014; Shpigel et al., 2018), new uses for their products should be explored as pharmaceutical drugs, nutraceuticals (Holdt and Kraan, 2011), and other such products.

6. Influence of abiotic factors on phenylalanine and tryptophan production in *Ulva* spp.

The presence of the EAA Phe and Trp in *Ulva* spp. (van der Wal et al., 2013; Bikker et al., 2016; Mata et al., 2016) presents the opportunity for their use as anxiolytics in fish farming. Evaluations of the influence of abiotic factors such as seawater temperature, pH, salinity, nitrate and phosphate concentrations, and irradiance on essential amino acid production by seaweeds for anxiolytic purposes will have great importance as a biotechnology tool. Benjama and Masniyom (2011) and Wells et al. (2017) noted that since environmental parameters influenced algal biosynthesis, they could obtain specific substances from culture macroalgae through the manipulation of certain abiotic factors.

To identify Phe synthesis patterns in *Ulva* spp., we constructed a matrix composed of geographic locations (latitude and longitude), environmental data, the concentrations of the EAA encountered, and the protein contents in the *Ulva* spp. cited in the articles. Only articles specifying the analytical methods used for Phe quantification and having similar protein contents were considered (to help guarantee statistical consistency); for that reason, Trp analyses were excluded due to the small number of appropriate references. The data matrix included environmental parameters known to influence algal metabolism (Hurd et al., 2014) [mean sea surface temperature – MSST, thermal amplitudes of sea – TAS, salinity, pH, nitrate (N) and phosphate (P) concentrations, and irradiance (PAR)] obtained from the BioOracle data set (Tyberghein et al., 2012). Principal component analysis (PCA) and simple and multiple regressions were performed using that matrix, considering a significance level of $p < .05$, using the Statistica program, version 7, StatSoft Inc.

A positive correlation was detected between Phe concentrations and mean sea surface temperatures (MSST), and a negative correlation with pH, independent of the species evaluated (Fig. 1). Regression analyses were significant for the isolated parameters ($\text{Phe} = -18.59 + 3.29 \times \text{MSST}$, $R^2 = 0.33$, $p = .004$, $\text{Phe} = 3990.89–481.12 \times \text{pH}$, $R^2 = 0.52$, $p = .0008$)

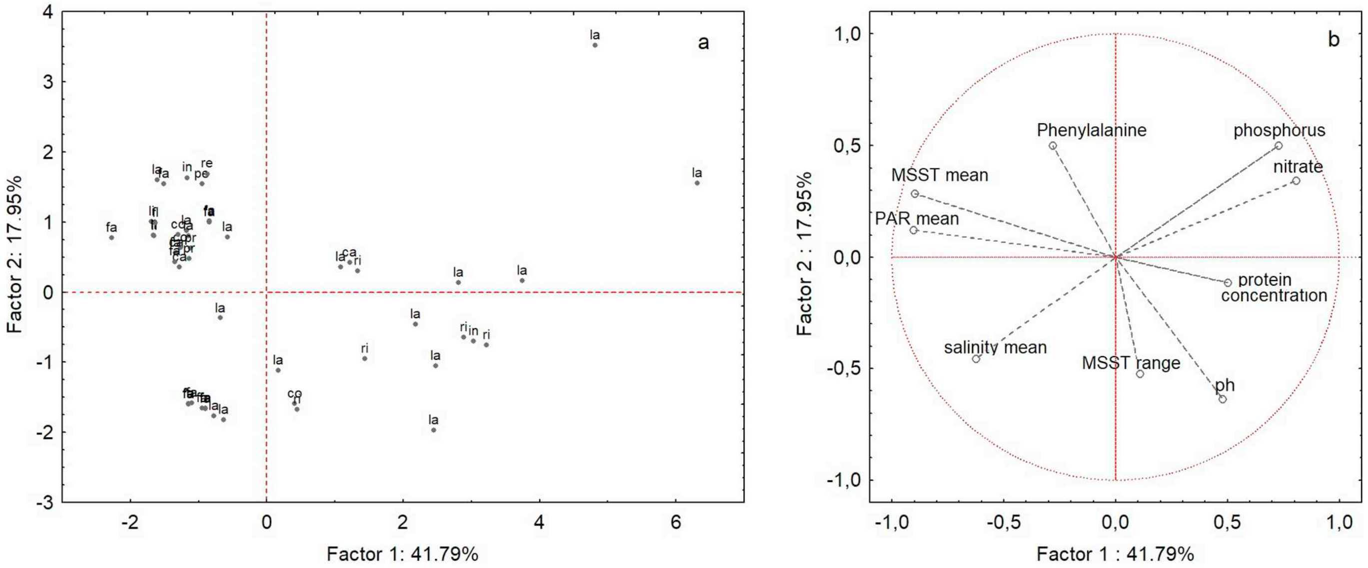

Fig. 1. Principal Components Analysis of the phenylalanine concentrations (Phe) in *Ulva* species, considering the abiotic factors of their origin.

but were more robust when multiple regression was used (Phe = 10,046.35–6.50 × MSST − 1202.93 × pH, $R^2 = 0.65$, $p = .017$). Limitations of the analyses related to a reduced number of cases, the standardization of the analytic methods among the articles, and the absence of detailed descriptions of the environmental characteristics of the sample's origin, did not allow the construction of models that could predict phenylalanine concentrations in *Ulva* species. We recommend that MSST and pH factors be prioritized in future studies that seek to increase EAA production in *Ulva* species.

Although *Ulva* species show wide tolerance ranges to some abiotic factors (e.g., salinity, temperature, luminosity, and nutrient concentrations) in relation of their growth, those factors can greatly influence the production of bioactive compounds (Benjama and Masniyom, 2011; Angell et al., 2015; Mata et al., 2016). At salinity levels of 45–60 PSU, for example, *U. ohnoi* had a low growth rate but produced large quantities of total amino acids (although salinity did not influence Phe concentrations) (Angell et al., 2015). The small numbers of reports for some species (Angell et al., 2015; Moustafa and Eladel, 2016), however, may have masked their differential responses to abiotic factors.

Interesting examples are found in the literature of the manipulation of temperature, light, and pH conditions to obtain desirable natural products from *Ulva* in aquaculture. The use of Recirculation Aquaculture Systems (RAS) made it possible to estimate the quantities of desired biochemical compounds produced by *U. ohnoi* during six months of cultivation, with a total protein yield of 18.4 t.ha^{-1}.year^{-1} and a 41% EAA content (Mata et al., 2016).

In addition to the possibility of obtaining accurate production estimates using RAS, the inclusion of *Ulva* in animal feed could benefit fish health through the inclusion of nutraceutical compounds, such as vitamin E and several EAA (Table 2). As half of all fish farm production costs are related to nutrition, the inclusion of *Ulva* in their feed (even if only partially) would reduce the amount of fishmeal needed, diminish pollution caused by that food source, and minimize environmental damage resulting from overfishing (Trushenski et al., 2010; Bleakley and Hayes, 2017; Quezada-Rodríguez and Fajer-Ávila, 2017).

Algae can be added to the feeds of both omnivorous and carnivorous fish without compromising zoo-technical parameters such as fish length and biomass, feed conversion efficiency, and blood parameters (Madibana et al., 2017). Algae can also replace traditional feeds in shrimp farming. *U. lactuca* successfully replaced commercial feed without compromising shrimp growth or its EAA composition in the culture of *Litopeneaus vannamei* (Boone) (Pallaoro et al., 2016).

7. Questions that need to be addressed in the future

Although Trp and Phe are found in *Ulva* and are useful as anxiolytics, there are no actual published citations of *Ulva* spp. being used as an anxiolytic source in any fish farm. Phe and Trp are obtained from other sources for that purpose. Will the inclusion of *Ulva* in fish feed provide anxiolytic effects?

As Phe and Trp are crucial to fish health as anxiolytics, and environmental factors influence their production by *Ulva*, the growth conditions of that algae could be manipulated to obtain higher EAA productions. Modeling using Phe data from *Ulva* spp. grown under different environmental conditions indicated that the principal factors governing high yields were mean sea surface temperatures and pH. The identification of the Phe synthesis patterns in *Ulva* spp. showed the importance of those abiotic factors to increasing EAA production in *Ulva* species; there was not sufficient data available to determine the factors influencing Trp production. What then are the factors that influence high Trp production? Data from the literature has shown that the responses of cultivated fish to supplementary Phe and Trp will vary according to the fish species and the amino acid concentrations used. Can a standard concentration be established for each fish species?

The lack of reports on Trp produced by *Ulva* spp. is related to the extraction method used, since Trp can only be detected in *Ulva* spp. when alkaline hydrolysis is employed. Due to the importance of that EAA to fish farming, its existence in *Ulva*, and the possibility of cultivating that algae (while manipulating environmental factors to increase EAA production), the question of which environmental factors can increase Trp production will be very important.

As it was possible to identify environmental factors cited in the literature that promoted the increase of Phe concentrations in *Ulva* spp., accurately detecting tryptophan in *Ulva* spp. will potentiate the use of that alga in different industrial sectors, such as pharmaceuticals, food production, etc. What then are the alternative methods of alkaline hydrolysis for detecting tryptophan in *Ulva* spp.?

We verified the potential use of *Ulva* spp. as anxiolytic sources for fish farming, although many issues remain to be addressed to perfect their use for that purpose.

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Corrigendum

Corrigendum to “*Ulva* spp. as a natural source of phenylalanine and tryptophan to be used as anxiolytics in fish farming” [Aquaculture 509 (2019) 171–177]

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The authors regret the corrections on the Table 1: In the first line of the first column change *Bricon amazonicus* (Spix & Agassiz, 1829) Matrinchã instead *Cirrhinus mrigala* (Hamilton, 1822) Mrigal carp. In the second column of the first line: change Bryconidae instead Cyprinidae.

Table 2: In the table caption change 2017 instead 2018.

Page 172, second paragraph, sixth line, in the item - 4. Phenylalanine and tryptophan in *Ulva* spp.: Please change “only two quantified Trp;” instead “only one quantified Trp;”.

The authors would like to apologise for any inconvenience caused.

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