

## INTEGRATION OF MACROALGAE IN FISH FARMING: A SUSTAINABLE APPROACH

### INTEGRAÇÃO DE MACROALGAS NA PISCICULTURA: UMA ABORDAGEM SUSTENTÁVEL

### INTEGRACIÓN DE MACROALGAS EN LA PISCICULTURA: UN ENFOQUE SOSTENIBLE



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#### ABSTRACT

Aquaculture has grown globally due to limitations in extractive fishing and the increasing demand for fish; however, intensive systems generate significant environmental impacts, especially due to nutrient accumulation and deterioration of water quality. The aim of this study was to conduct a meta-analysis of bibliographic data related to research on integrated fish and macroalgae farming systems, highlighting the strategic relevance of the topic in the international scenario and its emerging importance in the Brazilian Aquaculture context. Thirteen studies were selected, totaling 27 valid comparisons. The analysis, performed using multilevel models with  $\ln RR$  as the effect size, revealed that farming with macroalgae significantly increased fish growth ( $\ln RR = 0.1492$ ;  $p = 0.0184$ ), corresponding to an average increase of 16.1% compared to monoculture. The heterogeneity among the studies was significant, although partially explained by the evaluated moderators. Among the macroalgae species studied, *Gracilaria* sp. stood out with the most pronounced positive effect ( $p = 0.0025$ ), while the duration of the experiments did not significantly influence the results. The absence of publication bias reinforces the reliability of the estimates obtained. In general, the findings show that integrated systems with macroalgae contribute to improving water quality, especially by reducing total ammoniacal nitrogen (TAN) and other nitrogenous compounds, while also favoring fish growth (SGR), based on a systematic review that selected 13 studies, totaling 27 valid comparisons. The analysis, performed using multilevel models with  $\ln RR$  as the effect size, revealed that the present favors fish growth and increases the environmental and productive sustainability of fish farming. Despite these benefits, there is still a scarcity of studies, especially in tank systems and recirculating aquaculture systems (RAS), indicating the need for further, more standardized, and long-term research to consolidate the mechanisms involved and strengthen the ecological and economic potential of this approach.

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**Keywords:** Integrated Farming Systems. Macroalgae. Meta-Analysis. Fish Farming.

## RESUMO

A Aquicultura tem crescido globalmente devido à limitação da pesca extrativista e à crescente demanda por pescado, porém os sistemas intensivos geram impactos ambientais relevantes, especialmente pelo acúmulo de nutrientes e pela deterioração da qualidade da água. O objetivo deste estudo consistiu em realizar uma meta-análise de dados bibliográficos referentes a pesquisas sobre sistemas integrados de cultivo de peixes e macroalgas, destacando a relevância estratégica do tema no cenário internacional e sua importância emergente no contexto aquícola brasileiro. Foram selecionados 13 estudos, totalizando 27 comparações válidas. A análise, realizada por meio de modelos multiníveis utilizando InRR como tamanho de efeito, revelou que cultivos com a presença de macroalgas promoveram aumento significativo no crescimento dos peixes ( $\ln RR = 0,1492$ ;  $p = 0,0184$ ), correspondendo a um incremento médio de 16,1% em relação ao monocultivo. A heterogeneidade entre os estudos foi significativa, embora parcialmente explicada pelos moderadores avaliados. Entre as espécies de macroalgas estudadas, *Gracilaria* sp destacou-se com efeito positivo mais pronunciado ( $p = 0,0025$ ), enquanto a duração dos experimentos não influenciou significativamente os resultados. A ausência de viés de publicação, reforça a confiabilidade das estimativas obtidas. De modo geral, os achados evidenciam que sistemas integrados com macroalgas contribuem para a melhoria da qualidade da água, especialmente pela redução de nitrogênio total amoniacal (TAN) e outros compostos nitrogenados, ao mesmo tempo favorece o crescimento dos peixes (SGR), a partir de uma revisão sistemática que selecionou 13 estudos, totalizando 27 comparações válidas. A análise, realizada por meio de modelos multiníveis utilizando InRR como tamanho de efeito, revelou que o presente favorece o crescimento dos peixes e aumenta a sustentabilidade ambiental e produtiva da piscicultura. Apesar desses benefícios, ainda há escassez de estudos, especialmente em sistemas de tanques e sistemas de recirculação (RAS), indicando a necessidade de pesquisas adicionais, mais padronizadas e de longo prazo, para consolidar os mecanismos envolvidos e fortalecer o potencial ecológico e econômico dessa abordagem.

**Palavras-chave:** Sistemas de Cultivo Integrado. Macroalgas. Meta-Análise. Piscicultura.

## RESUMEN

La acuicultura ha crecido a nivel mundial debido a las limitaciones de la pesca extractiva y al aumento de la demanda de pescado; sin embargo, los sistemas intensivos generan impactos ambientales relevantes, especialmente por la acumulación de nutrientes y el deterioro de la calidad del agua. El objetivo de este estudio fue realizar un meta-análisis de datos bibliográficos provenientes de investigaciones sobre sistemas integrados de cultivo de peces y macroalgas, destacando la relevancia estratégica del tema en el escenario internacional y su importancia emergente en el contexto aquícola brasileño. Se seleccionaron trece estudios, totalizando 27 comparaciones válidas. El análisis, realizado mediante modelos multinivel utilizando InRR como tamaño del efecto, reveló que los cultivos con presencia de macroalgas promovieron un aumento significativo en el crecimiento de los peces ( $\ln RR = 0,1492$ ;  $p = 0,0184$ ), lo que corresponde a un incremento promedio del 16,1% en comparación con el monocultivo. La heterogeneidad entre los estudios fue significativa, aunque parcialmente explicada por los moderadores evaluados. Entre las especies de macroalgas estudiadas, *Gracilaria* sp. se destacó con un efecto positivo más pronunciado ( $p = 0,0025$ ), mientras que la duración de los experimentos no influyó significativamente en los resultados. La ausencia de sesgo de publicación refuerza la confiabilidad de las estimaciones obtenidas. En general, los hallazgos evidencian que los sistemas integrados con macroalgas contribuyen a la mejora de la calidad del agua, especialmente mediante la reducción del nitrógeno amoniacal total (TAN) y otros compuestos nitrogenados, al mismo

tiempo que favorecen el crecimiento de los peces (SGR). A pesar de estos beneficios, aún existe una escasez de estudios, especialmente en sistemas de tanques y sistemas de recirculación acuícola (RAS), lo que indica la necesidad de investigaciones adicionales, más estandarizadas y de largo plazo, para consolidar los mecanismos involucrados y fortalecer el potencial ecológico y económico de este enfoque.

**Palabras clave:** Sistemas de Cultivo Integrado. Macroalgas. Meta-Análisis. Piscicultura.

## 1 INTRODUCTION

Since the 1990s, Aquaculture has been consolidated as one of the fastest growing productive sectors in the world agricultural scenario, standing out for its growing capacity to meet the global demand for aquatic foods (Troell et al., 2009; Abreu et al., 2011; FAO, 2022). Data from the Food and Agriculture Organization (FAO) show that the cultivation of aquatic organisms showed the highest average growth rates between the years 2000 and 2016, remaining in 2020 as one of the main food products traded internationally, with about 60 million tons exported, excluding algae (FAO, 2018; Ahmed et al., 2019; FAO, 2022). The sector's advance is driven, above all, by the stabilization of extractive fishing, which has ceased to keep up with the increase in global demand, positioning Aquaculture as an essential alternative in a context of limited natural resources (Waite et al., 2014). In 2022, the sector reached a historic milestone by surpassing, for the first time, extractive fishing, registering approximately 94 million tons — about 51% of all production of aquatic organisms (Seafood, 2024; FAO, 2024).

World production is heavily concentrated in Asian countries, with China and Indonesia leading the way in marine cultivation since the 1990s. In Brazil, a country with vast aquaculture potential, shrimp farming, bivalve mollusk farming, and continental fish farming stand out as the main activities in operation (Nascimento et al., 2022). Despite the promising start in the 1930s, national marine fish farming remains little explored, reflecting structural, technological and coastal environment use planning challenges (Cavalli et al., 2009; FAO, 2022).

The expansion of fish farming—particularly those conducted in intensive monoculture systems—has been accompanied by a series of environmental impacts and production limitations (Naylor et al., 2000; Valiela et al., 2001). The accumulation of nutrients, especially nitrogen and phosphorus, can cause chemical changes in sediments, compromise the benthic community, and reduce local biodiversity (White et al. 2004; Naylor et al., 2005). Studies show that these effects deteriorate environmental quality, increase operating costs and increase economic risks for producers, especially due to dependence on external inputs and the vulnerability of the production system to environmental fluctuations (Cabelo 2006; Mazur & Curtis 2008; Bergqvist & Gunnarsson 2013; Kerrigan et al., 2018).

In the face of these challenges, alternative production models have been gaining prominence, especially Integrated Multitrophic Aquaculture (IMTA). Although species integration is an ancient practice, with millennial records in traditional Asian systems, the modern concept of IMTA gained notoriety in the early 2000s, driven by research that demonstrated its ability to mitigate the environmental impacts of intensive fish farming

(Chopin, 2001; Mirzoyan et al., 2010). In this system, cultivated species of different trophic levels interact in a complementary way: organic and inorganic extractive organisms take advantage of metabolic waste generated by fish, promoting bioremediation, economic diversification and greater production efficiency (Chopin et al., 2010). Thus, this integrated production system seeks to minimize the ecosystem impacts of monoculture, as well as to optimize fish production, reaching the growth potential of Aquaculture, and bringing a better financial cost-benefit (Rhyter et al., 1975; Neori et al., 2004).

Among the most promising extractive components, macroalgae occupy a prominent position (Castelar et al., 2014). Endowed with a high capacity for assimilating nutrients, these organisms act as true biological filters, removing nitrogenous compounds, stabilizing the pH and raising the dissolved oxygen in the water (Neori et al., 2004; Kang et al., 2012). As a consequence, they contribute to the improvement of water quality, the well-being of farmed fish, and the reduction of the system's environmental impacts (Araújo et al., 2018). In addition, they have low production costs and wide potential for economic use, whether in the food, pharmaceutical, cosmetic, biofuel or animal feed industries (Lal et al., 2005; Suutari et al., 2014). Rhodophytes are widely cultivated for the production of carrageenan and agar, while chlorophytes, although less produced, represent an important source of ulvan, a polysaccharide of growing industrial interest (Torres et al., 2019; Lakshmi, 2020).

Despite their advantages, integrated systems with macroalgae and fish still face operational and technological challenges, especially in open marine environments, where biomass control, the risk of escapes, and hydrodynamic variations make management difficult (Buschmann et al., 2001). In addition, regulatory limitations, the absence of specific regulations and the resistance of some producers, often associated with the lack of technical knowledge and the initial costs of adaptation, still restrict the commercial expansion of IMTA in several countries (Troell et al., 2003; Barrington et al., 2009; Granada et al., 2016).

In this context, understanding the development of Aquaculture, its challenges and the emerging sustainable alternatives is essential to guide the sector's transition towards more resilient, efficient and environmentally responsible production models. Integrated systems, especially those based on the incorporation of macroalgae, represent a promising strategy to reconcile economic production, sustainability and conservation of aquatic ecosystems.

The integrated system with macroalgae appears in the literature as an excellent alternative to mitigate or solve the main problems of intensive fish farming, being, however, a recent practice and with few studies that synthesize and compare the research elaborated on this theme. Thus, the objective of this study was to carry out a meta-analysis of bibliographic data related to research on integrated fish and macroalgae cultivation systems,

highlighting the strategic relevance of the theme in the international scenario and its emerging importance in the Brazilian aquaculture context.

## 2 MATERIALS & METHODS

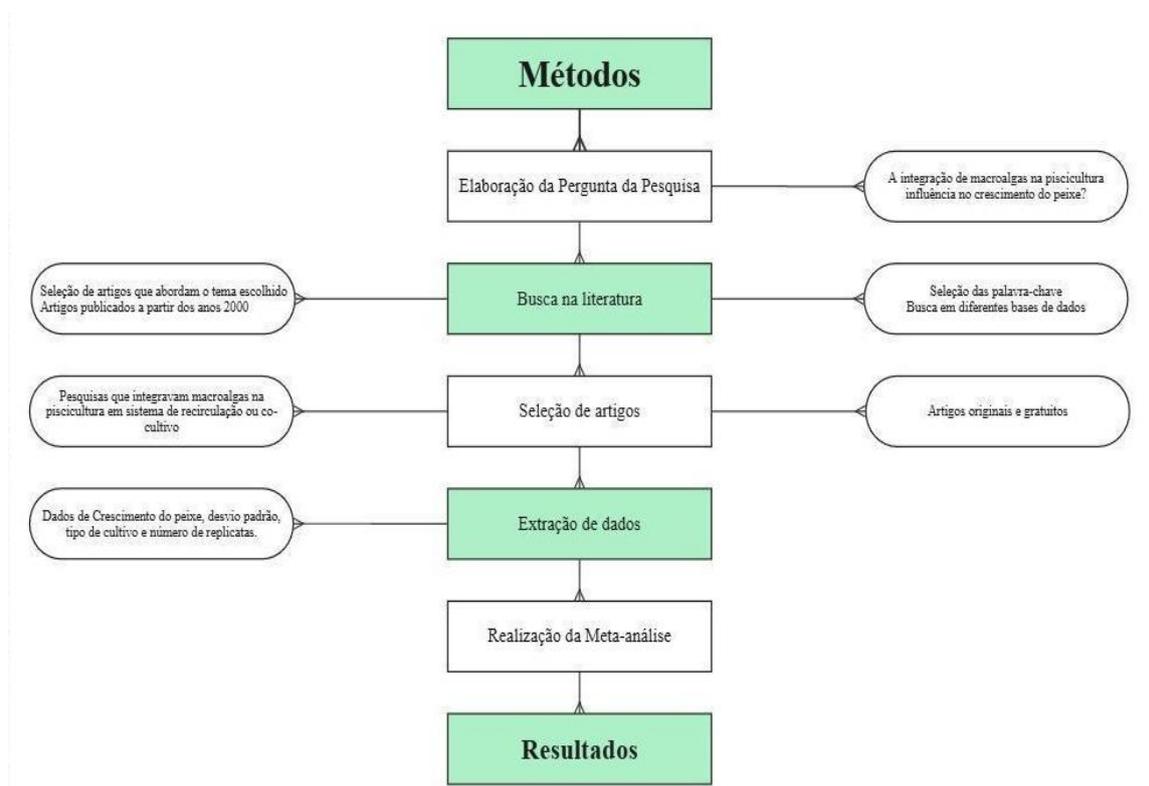
### 2.1 LITERATURE REVIEW AND DATA COMPILATION

A bibliographic database on integrated fish and macroalgae cultivation systems was carried out through Google Scholar (<https://scholar.google.com>), Science direct (<https://www.sciencedirect.com/>), Wiley (<https://onlinelibrary.wiley.com/>), Periodicos Capes and Academy (<https://www.periodicos.capes.gov.br/> and Academia.Edu). The following keywords were used: "IMTA"; "seaweed"; "TAN", "fish", "growth", "bioremediation" and "polyculture", selecting works written since the year 2000. The first 1000 publications retrieved from the survey were screened. In addition, an intense search was carried out in the bibliographic data of each article, to add works to the present study. The data were extracted only from scientific articles published and freely available. Gray literature was excluded from the database.

The studies with recirculating water (RAS) that reported the specific growth rate (SGR) of the fish, duration of the experiment (days), macroalgae and fish species, total ammonia nitrogen (or TAN) content, type of cultivation (polyculture or monoculture), experimental control conditions and number of replicates were compiled in a single database (Figure 1). Works that did not have one or more necessary information were excluded from the present study. In addition, authors were asked whenever these data were not available. To compose the database, the year of publication and the location, when available, were also inserted. Some studies presented more than one measured abiotic variable associated with water quality, which were not selected because they were not common to all publications.

**Figure 1**

Flowchart of methodology for selecting articles, determining the selection criteria



## 2.2 STATISTICAL ANALYSES

Statistical analysis was conducted according to the guidelines proposed by Harrer et al. (2021), in the manual *Doing Meta-Analysis in R*, using the R Core Team software (version 4.5.1; 2025) and the metafor package (Viechtbauer, 2010). The values absent standard deviation were imputed, based on the average coefficient of variation per fish species, given by the formula, according to the methodological recommendation of Higgins & Green (2008) and Nakagawa & Santos (2012), in order to avoid the loss of valid comparisons and reduce the bias associated with the exclusion of incomplete studies.

For each study included in the database, the log response ratio (lnRR) was calculated, which expresses the magnitude of the effect of the treatment in relation to the control (Hedges & Vevea, 2005; Lajeunesse, 2010). Expressed by, where  $\bar{X}^{\text{trat}}$  and  $\bar{X}^{\text{cont}}$ , are the means of the specific growth rate (SGR) of fish in the treatments with and without macroalgae respectively. The variance of lnRR was calculated based on the propagation of the error of the means and their respective standard deviations and sample sizes ( $n^{\text{Trat}}$  and  $n^{\text{Cont}}$ ), according to the classical formula of (Hedges et al, 1999): where is the standard deviation and the number of replicates. The formulas were applied using automatic functions available in the metafor package. Multilevel models with and without moderators were adjusted, using the Restricted Maximum Likelihood Estimator (REML), with a hierarchical structure of random

effects, considering the existence of multiple comparisons in the same study, allowing the variance between studies and within studies to be estimated separately (Konstantopoulos, 2011; Nakagawa & Santos, 2012).

The basic model can be expressed as  $y_{ij} = \mu + u_j + v_{ij} + \varepsilon_{ij}$ ; the  $y_{ij}$ , effect size observed in the  $j$ - comparison of the study  $\mu$  the global average effect represents the variance between studies  $u_j \sim N(0, \tau_{entre}^2)$  represents the variance between comparisons within the studies;  $v_{ij} \sim (0, \tau_{intra}^2)$  represents the variance between comparisons within the studies; is the sampling error associated with variance  $v_{lnRR}$ . The model with moderators (explanatory variables), corresponding to the algae species and the duration of the experiment, thus the model is expressed by  $y_{ij} = \beta_0 + \beta_1(\text{espécie de alga}) + \beta_2(\text{duração}) + u_j + v_{ij} + \varepsilon_{ij}$ , allowing to investigate the heterogeneity observed in the differences between the species used or in the time of the experiment.

Due to the limited interpretation of I2, it was decided to report the  $\tau^2$  values by level, as recommended by Konstantopoulos (2011) and Viechtbauer (2010). The Egger test (Egger et al., 1997) was used to investigate the presence of publication bias, selecting a simpler study comparison for later application of the `regtest` function, with the absence of significance indicating symmetry in the funnel graph and reducing the probability of publication bias. The prediction was obtained using the `predict()` function, with point estimates ( $\hat{\mu}$ ), standard error (SE), confidence intervals (CI) and prediction intervals (PI).

### 3 RESULTS

Most of the selected works were found on the Google Scholar platform. The other databases provided low return of works, both in general, with the use of keywords, and for the selection of articles following the specifications necessary for inclusion in the meta-analysis. The bibliographic references of the selected works also helped to quantitatively optimize this work (Figure 2).

**Figure 2**

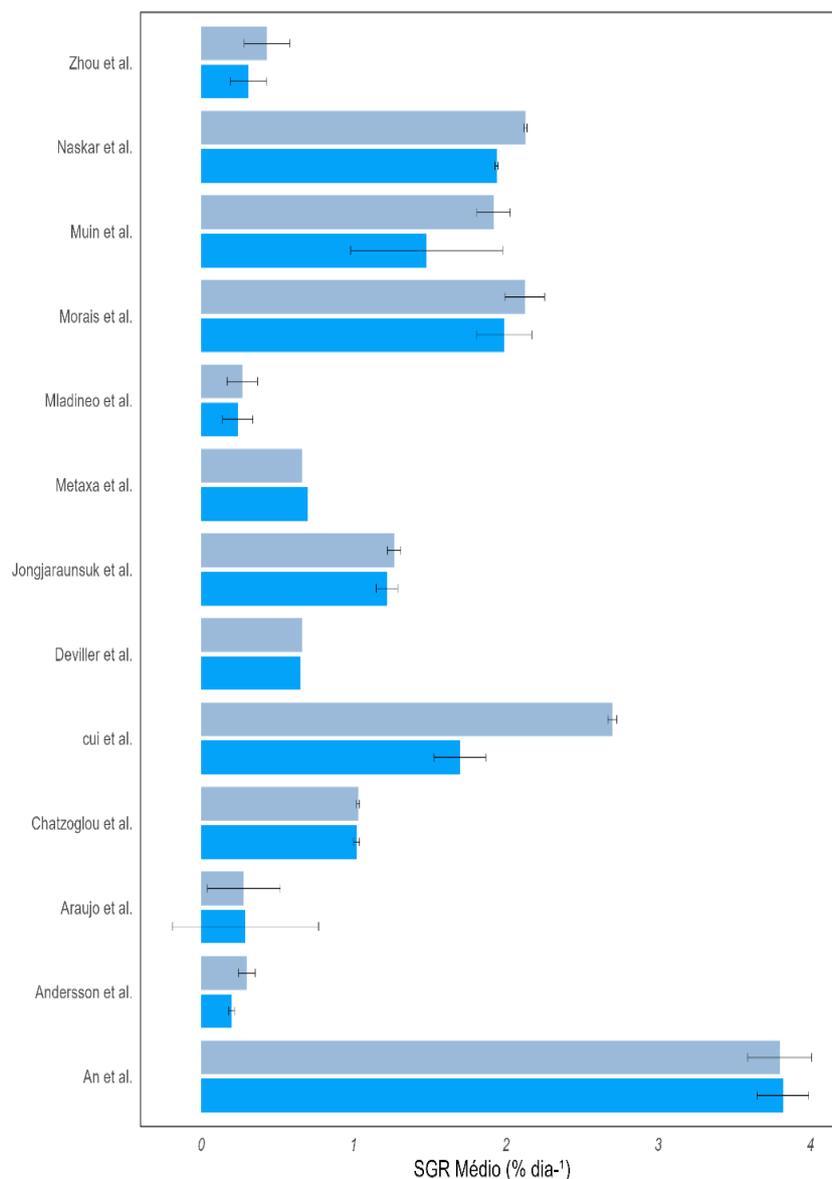
*Summary of the results found in online searches for experiments in published articles*



In all, only 13 studies were found that compared the breeding of at least one species of fish integrated to a species of macroalgae, with a control group, presenting data on the specific growth of the fish both in the control and in the experiment (Figure 3) and presenting culture in the same tank or in a recirculation system. Of these selected studies, 27 experiments were screened for use in the meta-analysis, presenting all the data necessary for their integration (Table 1).

**Figure 3**

Mean growth rate  $\pm$  standard deviation of fish (SGR) per author (means per replicates). Light blue = treatment; and dark blue = control

**Table 1**

Sorting of the data of the selected articles. The Mix algae species correspond to the treatments that used more than one algae species (*Ulva*, *Enteromorpha* and *Cladophora*)

Authors	Replicas	Duration of the Experiment	Fish Species	Algae Species	TAM (mg/L) Experiment	TAM (mg/L) Control
An & Anh (2020).	1	56	<i>Oreochromis niloticus</i>	<i>Gracilaria tenuistipitata</i>	0.70	1.53
Andersson et al. (2025)	2	126	<i>Oncorhynchus mykiss</i>	<i>Ulva fenestrata</i>		
Araújo et al. (2025)	1	65	<i>Sparus aurata</i>	<i>Ulva ohnoi</i>		
Chatzoglou et al., (2020)	1	84	<i>Dicentrarchus labrax</i>	<i>Ulva sp</i>	0.73	0.70

Cui et al. (2025)	1	30	<i>Pearl gentian grouper</i>	<i>Gracilaria sp</i>		
Deviller et al. (2004)	1	365	<i>Dicentrarchus labrax</i>	Mix	0.34	0.30
Jongjaraunsuk et al., (2025)	3	30	<i>Lates calcarifer</i>	<i>Caulerpa lentillifera</i>	0.70	1.20
Metaxa et al. (2006)	1	365	<i>Dicentrarchus labrax</i>	Mix	0.34	0.24
Mladineo et al. (2010)	1	365	<i>Dicentrarchus labrax</i>	<i>Chlorophyceae</i>	0.15	0.14
De Morais et al. (2023)	2	54	<i>Oreochromis niloticus</i>	<i>Ulva ohnoi</i>		
Muin et al. (2024)	2	30	Red Hybrid Tilapia	<i>Gracilaria changii</i>	0.71	2.17
Naskar et al. (2023)	3	60	<i>Chanos chanos,</i>	<i>Enteromorpha intestinalis</i>	0.58	0.77
Zhou et al. (2006)	8	35	<i>Sebastes fuscescens</i>	<i>Gracilaria lemaneiformis</i>	0.28	1.13

The adjusted multilevel model considered the dependence between comparisons of the same study, using a total of 27 comparisons and showed adequate convergence under the REML method. For the model, the random effects nested between the studies and between the comparisons within the same study were considered, evaluating the influence of the macroalgae species and the duration of the experiment (in days) on the combined effect of integrated fish and macroalgae cultivation.

Initially, a multilevel meta-analysis (model without moderators) was conducted to estimate the overall effect size, considering the hierarchical structure of the data. The analysis revealed an estimated overall effect size LnRR of 0.1492 (SE = 0.0593), which was statistically significant ( $z = 2.51$ ;  $p = 0.0184$ ; 95% CI [0.0272; 0.2712]; AIC = 0.0101; BIC = 3.8). This result indicates that, in general, the experimental treatment promoted a significant increase in the fish-specific growth rate, with an average increase of about 16.1% in the macroalgae group compared to the control group (since positive LnRR indicates an increase) (Viechtbauer, 2010). Cochran's heterogeneity test indicated significant heterogeneity between the studies ( $Q(26) = 196.09$ ;  $p < 0.0001$ ). The residual variability between studies ( $= 0.0249$ ) was considerably higher than the variability between comparisons within the same study ( $= 0.0017$ ). This suggests that methodological or biological differences between studies contribute more to the observed heterogeneity than variations within the same experiment.  $\tau_{\text{entre}}^2 \tau_{\text{intra}}^2$

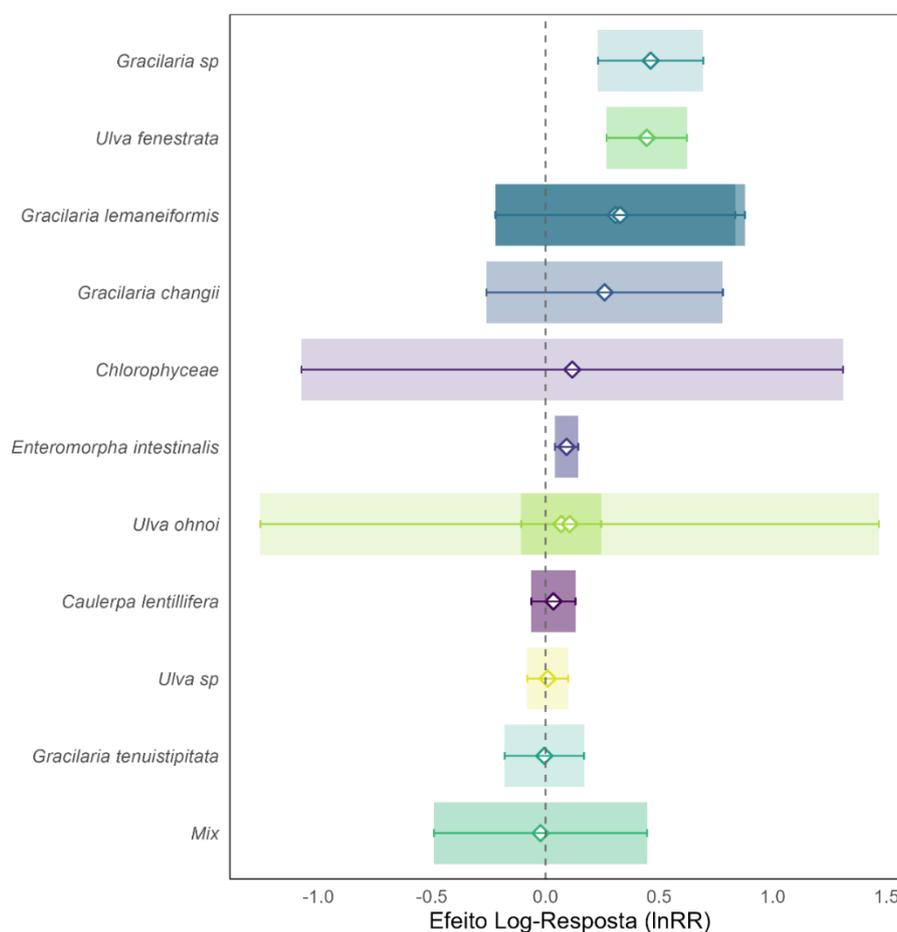
In order to investigate the sources of this heterogeneity, a multilevel meta-regression model was adjusted including the moderating variables "Macroalgae Species" and "Duration of the Experiment" (days). The test for the moderators was statistically significant ( $F(11,15) = 3,530$ ;  $p = 0.0128$ ), indicating that the variables included explain a significant portion of the

variability of the effect sizes. Residual heterogeneity remained significant ( $QE(15) = 100.1$ ;  $p < 0.0001$ ), suggesting the existence of other unmeasured factors that still influence the results.

In the analysis of the individual coefficients of the meta-regression, the continuous variable referring to the duration of the experiment did not present statistical significance ( $\tau^2 = 0.0034$ ;  $p = 0.9542$ ), indicating that the exposure time, within the analyzed interval, was not a determinant linear predictor for the variation in the size of the effect. On the other hand, the categorical analysis of the macroalgae species revealed specific differences. *Gracilaria* sp. showed a positive and highly significant effect ( $\tau^2 = 0.4279$ ;  $p = 0.0025$ ; 95% CI [0.1767; 0.6791]), which corresponds to a mean increase of about 53.4% in treatment compared to control. The other species and consortia did not present statistically significant differences in relation to the baseline level of the model ( $p > 0.05$  for the other coefficients), although there were wide confidence intervals for categories with lower sample representativeness (Figure 4). On the other hand, the ratio between the highest and lowest sampling variance was extremely large, resulting in unstable results. This was confirmed in the result for the Akaike model (AIC = 17.34) and the Bayesian Information Criterion (BIC= 27.25) which are significantly higher for this model, which indicates an unfavorable performance.

**Figure 4**

Standardized effects by algae species. Each point represents the estimated mean effect (lnRR). The error bars are the confidence intervals (95% CI). The overlapping of colors indicates the contribution of further studies for each species. Mix = treatments that used more than one species of algae (*Ulva*, *Enteromorpha* and *Cladophora*)

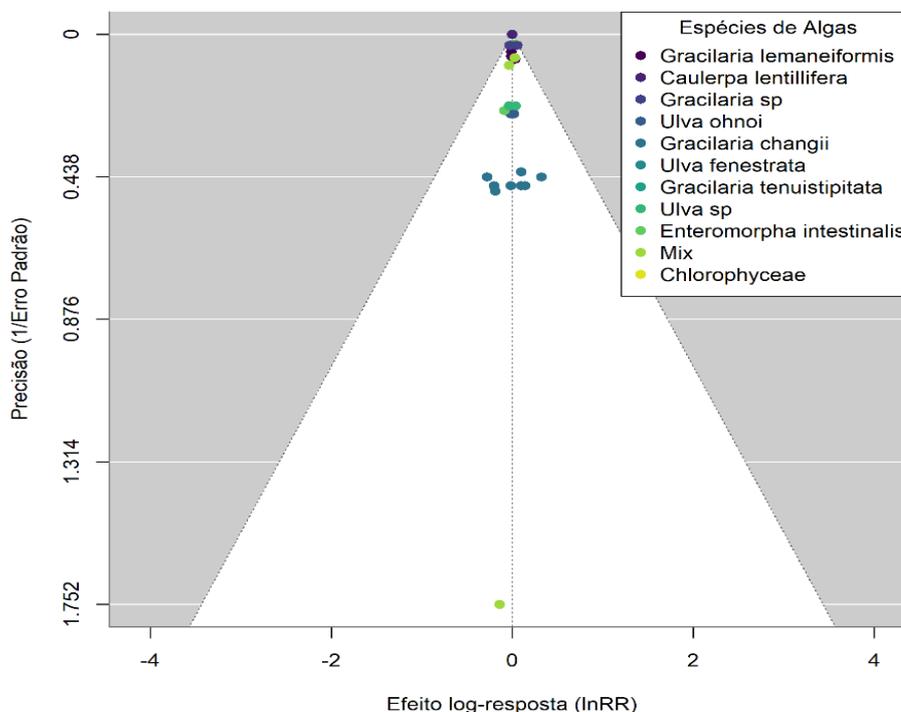


In this moderator analysis, the residual variability between studies was nil ( $\tau_{entre}^2 = 0$ ) relative to the variability between comparisons within the same study ( $\tau_{intra}^2 = 0.04$ ). This suggests that, after the inclusion of the moderators, there was no further variation between the studies analyzed.

The Egger test, modified for multilevel models, evaluated the robustness of the results in terms of publication bias, using a representative comparison per study to ensure data independence. The test did not show significant asymmetry in the funnel graph ( $z = 0.2079$ ;  $p = 0.8353$ ), which suggests the absence of detectable publication bias in this sample of studies (Figure 5). This result reinforces the symmetry in the distribution of the studies and the robustness of the estimates obtained, suggesting that there is no evidence of publication bias among the studies included in the meta-analysis (Egger et al., 1997).

**Figure 5**

*Funnel Chart - Asymmetry Assessment. Dots represent an effect size as a function of their standard error (precision). The symmetrical shape of the funnel around the centerline indicates the absence of publication bias. Mix = treatments that used more than one species of algae (Ulva, Enteromorpha and Cladophora)*



## 4 DISCUSSION

In general, the results reveal that the presence of macroalgae in fish farming systems promotes positive and consistent effects, especially through ecological mechanisms of synergy and biofiltration, such as those presented in Table 1 (Neori et al., 2004). From the synthesis of the data and the marked reduction in total ammonia nitrogen (TAN) levels, it is possible to infer that the fish-macroalgae interaction plays a central role in optimizing productivity in integrated systems (Chopin et al., 2001). The increases in fish-specific growth rate (SGR) observed in the studies are attributed to the improvement in water quality due to efficient nutrient recycling mediated by macroalgae (Neori et al., 2004; Chopin et al., 2001; Msuya & Neori, 2008; Neori et al., 2004).

Macroalgae use the nitrogenous excreta of fish as a source of nutrition, converting these compounds into their own biomass (Ramli et al., 2020). This internal nutrient cycle reduces nitrogen loss to the system, while improving overall energy efficiency, as less energy is wasted in detoxification processes or oxidative stress caused by unused ammonia (Troell et al., 2003). For example, the classic study by Zhou et al. (2006) showed that *Gracilaria*

*G. lemaneiformis* integrated with the cultivation of *Sebastodes fuscescens* in coastal waters, can remove significant amounts of dissolved nitrogen and phosphorus, functioning as an efficient "nutrient pommel", reducing the waste load and contributing to the ecological sustainability of the system. Additionally, macroalgae perform a crucial ecosystem service in the physicochemical stabilization of the water column. Through photosynthesis, they promote the sequestration of dissolved CO<sub>2</sub> and oxygen supplementation, acting directly in pH buffering and mitigating hypoxia or acidification of water (Msuya & Neori, 2008). This environmental stability reduces the metabolic cost associated with osmoregulation and acid-base balance of fish (Barrington et al., 2009). By minimizing energy expenditure in compensatory responses to respiratory stress, a more efficient allocation of resources for somatic growth and immune integrity is observed (Barrington et al., 2009). Therefore, the greater biological efficiency in integrated systems does not only result from decontamination, but also from the reduction of environmental fluctuations that required constant homeostatic adjustments.

The quantitative analysis of the data (Figure 3) corroborates the efficiency of bioremediation in mitigating nitrogen toxicity. A drastic reduction in total ammonia nitrogen (TAN) concentrations is observed in integrated systems, which is exemplified by the cultivation of macroalgae. A similar pattern has been documented for *Lates calcarifer* integrated with *Caulerpa lentillifera*, indicating an absorption capacity that prevents the accumulation of nitrogenous compounds at lethal or sublethal levels (Chaitanawisuti et al., 2011). In addition, the cultivation of macroalgae can bring indirect ecological benefits: for example, studies have shown that the presence of *G. lemaneiformis* can increase plankton biodiversity and modulate the microbial community in coastal systems, which can favor a more stable and balanced aquatic environment (Chai et al., 2018). Therefore, the presence of macroalgae in their experiments, reflected in total ammonia nitrogen (TAN) reductions and relatively low standard deviations in some control points, is consistent with the literature that reinforces the ecological value of integrated systems.

The values of the fish-specific growth rate (SGR) observed in figure 3, on the other hand, vary greatly. This variability may reflect methodological differences between studies (e.g. in algae density, not incorporated here) or in the culture mode (IMTA, RAS, bioflocs), in fish species, and in the duration of the experiments. Studies such as De Morais et al. (2023), show that the density of *Ulva ohnoi* in biofloc systems can influence both nutrient recovery and total productivity. The authors compared three treatments (without algae, 1 g·L<sup>-1</sup> e 2 g·L<sup>-1</sup> of *U. ohnoi*) and observed that both treatments with algae promoted greater nitrogen and phosphorus recovery, in addition to increased overall productivity, without significant impairment in fish and shrimp growth (De Morais et al., 2023). The physiological relevance

of this removal lies in the prevention of histopathological and metabolic damage. Chronic exposure, even at moderate ammonia levels, induces branchial hyperplasia, impairs gas exchange, and inhibits central nervous system enzymes, resulting in seizures and appetite suppression (Ip & Chew, 2010). Therefore, the presence of macroalgae acts as a toxicological barrier, keeping water quality within physiological tolerance limits and ensuring the osmoregulatory integrity of fish.

The bioremediation role of macroalgae extends to the management of the balance of nutrients essential for the ecological health of the system. Empirical evidence, such as the work carried out in Brazil by (Castelar et al., 2015), demonstrates that species such as *U. fasciata* and *G. birdiae* are able to efficiently remove ammonia and nitrate from effluents, reaching rates of more than 98% of ammonia removal in a five-day cycle in tanks. These authors also emphasize that the simultaneous assimilation of phosphate by these macroalgae contributes to the maintenance of a healthy nutritional balance in the effluent (Neori et al., 2004). This extraction of multiple nutrients prevents excessive accumulation that triggers eutrophication, the proliferation of unwanted microalgae and extreme oxygen fluctuations in the environment (Neori et al., 2004), ensuring ecological stability and reducing the environmental impact of the integrated system (Buschmann et al., 2008).

Within the premises of a quantitative review with a moderate number of comparisons ( $k=27$ ) involving different species, the results of the meta-analysis are considered statistically consistent. As evidence to support this robustness, Egger's alternative test can be considered, which did not indicate asymmetry in the funnel. This suggests that the results are not inflated by publication bias, allowing greater confidence in the estimates obtained (Egger et al., 1997). Another factor indicates the low variance between studies, reinforcing the good calibration of the effects within the samples carried out. Among the factors, the set of moderators "macroalgae species + duration" was significant, indicating that the model has real explanatory capacity, and not just noise. Corroborating this, the average effect ( $\pm 16\%$ ) suggests a positive overall trend without exaggeration (Cohen, 2013), with the amplitude of the interval being aligned with the sample size (Borenstein et al., 2021). This pattern is common in ecological reviews when few categories show truly high effects (Koricheva; Gurevitch; Mengersen, 2013), while the others remain close to neutrality. In multilevel meta-analysis models, such behavior suggests the presence of biologically relevant moderators influencing specific responses (Konstantopoulos, 2011).

The absence of a significant effect of the duration of the experiments indicates that the exposure time, within the range included in the studies, did not systematically contribute to explain the magnitude of the results. This absence of temporal trend is common in ecological

syntheses and usually indicates that physiological and ecological responses tend to stabilize rapidly (Gurevitch et al., 2018), or that studies use similar temporal windows (Koricheva et al., 2013).

The strongly significant result found for the species *Gracilaria* sp and the absence of effect for other species is an expected pattern when few macroalgae have really high effects, or when species treated as categories show marked ecological differences (Bokhtiar et al., 2022; Li-Hong et al., 2002). In summary, this suggests that, although the average effect is positive, the magnitude of this result is not uniform across species or studies. The low inter-study variance ( $\tau^2 \approx 0$ ) indicates that most of the detected variability is attributed to the differences within the studies themselves, which is consistent with controlled and reproducible experimental structures (Bokhtiar et al., 2022; Patterson & Thompson, 1971). The highly significant effect observed for *Gracilaria* sp demonstrates that this genus exerts a disproportionately greater impact when compared to the other macroalgae species analyzed here. For example, Wang et al. (2014) showed that the species *Gracilaria tenuistipitata* has a high growth rate under moderate concentrations of salinity (12–20) and high levels of inorganic nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), in addition to a marked preference for  $\text{NH}_4^+$ , which helps explain its ability to form blooms in estuarine environments. In addition, there is evidence that the experimental results for this genus are quite reproducible in different controlled studies. In cultivation on the coast of Cox's Bazar (Bangladesh), Bokhtiar et al. (2022), found systematic ways to optimize yield through manipulation of the lunar cycle, harvest interval, type of crop row, and sowing spacing.

The analysis of the data, reinforced by the specialized literature, highlights crucial methodological challenges that limit the generalization of the results of studies in integrated systems. First, the experimental design often lacks sufficient replicates or adequate cultivation scales for the intrinsic complexity of the system. This low replication compromises statistical validity and extrapolation, especially due to the great intrinsic variability between tanks, resulting from the complex interaction of organisms, nutrients, and microenvironments (Buschmann et al., 2001). One of the main critical limitations is the duration of the experiments, which in the review ranges from 30 to 365 days. Short-term studies can capture rapid improvements in total ammonia nitrogen (TAN) without, however, elucidating the long-term effects, such as nutrient saturation or competition for light and space, that influence the biomass allocation of algae.

Finally, the taxonomic variability among macroalgae is a determining factor for the heterogeneity of the results of fish-specific growth rate (SGR) and total ammonia nitrogen (TAN). Species such as *Gracilaria* sp and *Ulva* sp demonstrate different nutrient uptake

kinetics (e.g. different affinities for ammonia and nitrate) which requires a more robust methodological standardization to optimize nutrient extraction and maximize the growth of cultured organisms (Neori & Msuya, 2008). However, this review showed that there must be a good selection of species to be included in integrated cultivation systems, especially of macroalgae. It is known that each group has a better uptake of nutrients. In addition, the influences of different biomasses among the macroalgae could not be evaluated, which may evidence the need for different initial biomasses for each type of macroalgae in order to optimize their functions. Thus, studies that evaluate which species of macroalgae are more specifically indicated to be cultivated with certain species of fish become extremely important.

## **5 CONCLUSION**

The integrated analysis of empirical data and specialized literature confirms that integrated cropping systems constitute a functional and sustainable advance compared to traditional monoculture models. The inclusion of macroalgae contributes decisively to the mitigation of nitrogen toxicity and to the stabilization of physicochemical parameters, by converting metabolic by-products into useful biomass and closing essential biogeochemical cycles. In physiological terms, this more balanced environment favors greater biological efficiency and higher growth rates, since it reduces the energy demand for homeostatic maintenance and allows greater investment in anabolism. However, the consolidation of this technology still faces interpretative challenges. The heterogeneity of the statistical results, added to recurrent methodological limitations — such as low experimental replication, large temporal amplitude between studies, and high interspecific variability in the kinetics of nutrient uptake — highlights the need for standardization in research protocols. Thus, future investigations should prioritize long-term studies and pilot-scale experiments, in order to improve carrying capacity models and ensure that the observed ecological feasibility translates into productive and economic stability.

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