RESEARCH ARTICLE

Effect of dietary protein level on growth, food utilization, food conversion and survival rate of giant trevally (*Caranx ignobilis*)

[version 1; peer review: 2 not approved]

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Abstract

Background: Proper feed formulation is required for successful fish farming activities. Therefore, it is necessary for fish feed to provide optimal growth so that the cultivation business generates profits. Currently, there is very limited information about the appropriate feed for *Caranx ignobilis*, causing problems with its development. This study aims to provide feed with different protein levels to *C. ignobilis*.

Methods: We will examine the protein levels' effects on the daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency (FE), and survival rate (SR). This research was conducted for 35 days, from June to October 2017, at the Center Brackiswater Aquaculture Development (BPBAP) Ujung Batee, Ministry of Marine Affairs and Fisheries, Aceh Besar, Indonesia. This study used a completely randomized design method, with five treatment levels (30%, 40%, 50%, 60%, and 70% protein feed) and four replications.

Results: The results showed that feeding with different proteins on *C. ignobilis* had a significant effect on the mean values of DGR, SGR, AGR, FCR, FE, and SR. The 50% protein feed gave the best results for *C. ignobilis*, with a mean DGR value of 0.267 ± 0.005 g / day, a mean SGR of $1.722 \pm 0.030\%$ / day, a mean AGR of 0.081 ± 0.003 cm/day, a mean FCR of 1.290, a mean FE 77.755% and a mean SR was 86.667%.

Conclusions: Furthermore, feed treatment with increased protein

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content between 30%–50% has a positive correlation with the growth of *C. ignobilis*. However, the ability to grow fish will decrease if the feed protein content is >50%.

Keywords

Caranx ignobilis, protein, growth, ratio, efficiency

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Introduction

Giant trevally (*Caranx ignobilis*) is a reef fish species with a high economic value¹ because it is in very high demand in domestic and international markets, with a price range of IDR 42,000–80,000 / kg^{2,3}. Furthermore, *C. ignobilis* does not have muscles between muscles, so it is easy to consume⁴. Based on these advantages, *C. ignobilis* is exploited for consumption, economy, and recreation in various ways, including the use of destructive fishing gear (include e.g. bottom trawling, cyanide fishing, dynamite fishing, and ghost fishing)⁵. Massive coral damage due to environmental damage and the effects of global warming also puts pressure on the species population⁶. Therefore, it is necessary to conserve *C. ignobilis* through the domestication process so that numbers caught in nature can be reduced by diverting fishermen to *C. ignobilis* cultivation activities.

Fish that are eligible to become cultivated commodities must meet criteria such as economic value, desirability, fast growth, resistance to disease, and ease of domestication^{7–10}. *C. ignobilis* has several advantages, such as being euryhaline so that it can live in a salinity range of 15–32 ppt¹¹, it can live in conditions of high density (up to 150 individuals / m²¹²), fast growth¹³, economic value and no history of disease⁹. Based on these advantages, *C. ignobilis* can be cultivated in ponds and floating net cages and several fishermen in Aceh, Indonesia, have started cultivating this species in with this method¹⁴. However, there are several obstacles in developing cultivation, such as a lack of availability of commercial feed with the appropriate protein content. Therefore, we need a study on feed protein that can provide optimal growth in this fish species.

In cultivation activities, growth is a determining factor for success. The problem that is often faced by fish farming is the low value of protein, so cultivating fish is not optimal. According to Subandiyono and Hastuti¹⁵, protein has various roles and functions, including brave protein as a body structure, such as collagen, which is a fibrous connective tissue and has a solid structure to form fish muscles. Fish can grow optimally if protein needs are met. Protein has a role in determining the fish growth process because most of the fish's body (45–75% dry weight) consists of protein¹⁶. Also, protein is an efficient source of energy for aquatic animals, especially carnivorous fish¹⁷.

Methods

Time and place

This research was conducted for 35 days, from June to October 2017, at the Center Brackiswater Aquaculture Development (BPBAP) Ujung Batee, Ministry of Marine Affairs and Fisheries, Aceh Besar, Indonesia.

Research procedure

This study used a completely randomized design method, with five treatments and four replications. Fish were given experimental feed containing 30–70% protein as follows:

A = Treatment of feed containing protein 30%

B = Treatment of feed containing protein 40%

C = Treatment of feed containing protein 50%

D = Treatment of feed containing protein 60%

E = Treatment of feed containing protein 70%

Fish sample

This article is reported according to the ARRIVE guidelines. All efforts were made to lessen harm to the animals by complying to the guidelines of ethical animal use in research of Syiah Kuala University (Ethic Code No. 958/2015) and this study was approved by the Animal Research Ethics Committee of the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University (Ethic Code No. 63/KEPH/X/2020).

We obtained 300 *C. ignobilis* juveniles from Lancang Barat Village, Dewantara District, Aceh Utara Regency. The fish were acclimatized for two weeks at the BBAP Ujung Batee, Aceh Besar Indonesia. They were placed in 20 rearing nets of 1 × 1 meter in size, which were placed in a concrete pond containing 40 tons of water (range of salinity value 29.19–29.44‰), each containing 15 *C. ignobilis* juveniles.

Feed preparation

The experimental feed was made from fish meal, rebon shrimp flour, rice flour, soybean flour, cornflour, blood meal, coconut oil, CaCO₃, Isolesin, L-Tryptophan, DL-Methionine, and premix with a feed protein content of 30–70%. All ingredients were mixed and analyzed for protein content (Table 1).

Feeding procedure

Juvenile *C. ignobilis* were selected randomly. Their average weight was 11.41 ± 0.40 g and their average initial total length was 7.70 ± 0.76 cm. Furthermore, the fish are distributed into 20 nets measuring 1 × 1 meter. The net is placed in a concrete pond with a water volume of 40 tons (15 fish/net). The fish were given experimental feed twice a day at 7 am and 5 pm for 35 days, as much as 3% per body weight.

Research parameters

The parameters measured in this study are DGR, specific growth rate (SGR), AGR, feed conversion ratio (FCR), FE, and survival (SR). DGR and SGR were analyzed based on the formula by Muchlisin *et al.*^{18,19}. AGR was analyzed based on the Jones²⁰ formula, FCR, and FE were analyzed based on the formula of Shapawi *et al.*²¹; SR was analyzed based on the formula of Hseu *et al.*²².

$$\text{DGR (g day}^{-1}\text{)} = (\text{Wt} - \text{Wo}) / t,$$

$$\text{SGR (\% day}^{-1}\text{)} = [(\text{Ln Wt} - \text{Ln Wo})] / t \times 100,$$

$$\text{AGR (cm day}^{-1}\text{)} = \frac{L2 - L1}{\Delta t}$$

$$\text{FCR} = F / (\text{Wt} - \text{Wo})$$

$$\text{FE} = \frac{1}{\text{FCR}} \times 100 \%$$

$$\text{SR} = \frac{(\text{Nt})}{\text{No}} \times 100$$

Table 1. Feed formulation (g kg⁻¹) used in the study with a protein content of 30–70%.

Feed Ingredients	Protein Content Ingredients (%)	Feed Formulation (g)				
		Treatment				
		A	B	C	D	E
Rebon shrimp flour	58.80	20	20	20	20	20
Fish flour	59.00	410	620	660	500	140
Rice flour	7.26	480	250	90	10	10
Soybean flour	45.06	10	10	10	10	10
Corn flour	6.48	10	10	10	10	10
Blood flour	75.97	10	30	150	390	750
Coconut oil	0	5	5	5	5	5
CaCO ₃	0	5	5	5	5	5
Isoleucine	100	10	10	10	10	10
L-Tryptophan	100	17.5	17.5	17.5	17.5	17.5
DL-Methionine	100	17.5	17.5	17.5	17.5	17.5
Premix	0	5	5	5	5	5
Total (g)		1000	1000	1000	1000	1000
Protein (%)		30	40	50	60	70

Note: Wo is the initial weight of fish when researched (g); Wt is the weight of the fish at the end of the study (g); t is the study duration (days). L1 is the length of the fish at the start of raising (cm), L2 is the length of the fish at the end of raising (cm), Δt is the length of rearing (days). Nt is the number of test fish at the end of maintenance, and No is the number of test fish at the beginning of rearing.

Data analysis

Data of DGR, SGR, AGR, FCR, FE, and SR were analyzed statistically by one way ANOVA (Analysis of Variance) with a 95% degree of confidence ($P < 0.05$) using SPSS version 20 software. Significant data were tested further with Duncan's multiple ranges test.

Results

The results showed that diets with different protein content significantly affected ($P < 0.05$) the values of DGR, SGR, and AGR of *C. ignobilis*. The best growth of *C. ignobilis* was found in the feed treatment with 50% protein content, obtaining a mean DGR value of 0.27 ± 0.01 g / day, a mean SGR of $1.72 \pm 0.03\%$ / day and a mean AGR of 0.013 ± 0.01 cm / day, followed by 40% protein feed (mean DGR value 0.23 ± 0.01 g / day, SGR $1.50 \pm 0.04\%$ / day, and AGR 0.11 ± 0.01 cm / day), 30% protein feed (mean DGR value of 0.21 ± 0.01 g / day,

SGR $1.40 \pm 0.07\%$ / day, and AGR 0.08 ± 0.00 cm / day), protein feed 60% (mean DGR value 0.13 ± 0.01 g / day, SGR $0.98 \pm 0.07\%$ / day and AGR 0.07 ± 0.01 cm / day), and 70% protein feed (mean DGR value 0.12 ± 0.01 g / day, SGR $0.91 \pm 0.08\%$ / day and an AGR value of 0.07 ± 0.02 cm / day) (Table 2).

Furthermore, the results showed that the feed formulations with different protein content also significantly affected ($P < 0.05$) the value of FCR, FE, and SR of *C. ignobilis*. The best mean FCR, FE and SR values were shown in the 50% protein feed treatment with mean values of 1.29 ± 0.07 , $77.76 \pm 4.46\%$ and $86.67 \pm 5.44\%$ respectively, then followed by treatment of 40% protein feed (mean FCR 1.49 ± 0.17 , FE $67.73 \pm 7.61\%$, and SR $80 \pm 5.45\%$), 30% protein feed (mean FCR 1.66 ± 0.09 , FE $60.33 \pm 3.38\%$, and SR $78.33 \pm 3.34\%$), 60% protein feed (mean FCR 1.79 ± 0.06 , FE $56.03 \pm 1.84\%$, and SR $68.34 \pm 3.33\%$), and protein feed 70% (mean FCR 1.96 ± 0.15 , FE $51.19 \pm 3.71\%$, and SR $65.00 \pm 3.34\%$) (Table 3).

Discussion

The analysis showed that *C. ignobilis* growth occurred optimally with 50% protein feed based on the mean values of DGR, SGR, and AGR. Similar results were also found in its sister, *C. melampygus*; feed with about the same protein content (45%) provided optimum growth in these fish²³. Furthermore,

Table 2. Daily growth rate (DGR), specific growth rate (SGR), and absolute growth rate (AGR) of juvenile *Caranx ignobilis*.

Treatment	DGR (g / day)	SGR (% / day)	AGR (cm / day)
A	0.21 ± 0.01 ^b	1.40 ± 0.07 ^b	0.08 ± 0.00 ^a
B	0.23 ± 0.01 ^c	1.50 ± 0.04 ^c	0.11 ± 0.01 ^b
C	0.27 ± 0.01 ^d	1.72 ± 0.03 ^d	0.13 ± 0.01 ^c
D	0.13 ± 0.01 ^a	0.98 ± 0.07 ^a	0.07 ± 0.01 ^a
E	0.12 ± 0.01 ^a	0.91 ± 0.08 ^a	0.07 ± 0.02 ^a

Note: Numbers followed by different superscripts of letters indicate significant differences ($P < 0.05$). Numbers followed by a superscript of the same letter show no significant difference ($P > 0.05$). [A] 30% protein feed, [B] 40% protein feed, [C] 50% protein feed, [D] 60% protein feed, [E] 70% protein feed.

Table 3. The values of feed conversion ratio (FCR), feed efficiency (FE), and viability (SR) of juvenile *Caranx ignobilis*.

Treatment	FCR	FE (%)	SR (%)
A	1.66 ± 0.09 ^{bc}	60.33 ± 3.38 ^{bc}	78.33 ± 3.34 ^b
B	1.49 ± 0.17 ^b	67.73 ± 7.61 ^b	80.00 ± 5.45 ^b
C	1.29 ± 0.07 ^a	77.76 ± 4.46 ^a	86.67 ± 5.44 ^c
d	1.79 ± 0.06 ^{cd}	56.03 ± 1.84 ^{cd}	68.34 ± 3.33 ^a
E	1.96 ± 0.15 ^d	51.19 ± 3.71 ^d	65.00 ± 3.34 ^a

Note: Numbers followed by different superscripts of letters indicate significant differences ($P < 0.05$). Numbers followed by superscript of the same letter show no significant difference ($P > 0.05$). [A] 30% protein feed, [B] 40% protein feed, [C] 50% protein feed, [D] 60% protein feed, [E] 70% protein feed.

this study revealed that the increased feed protein content had a positive correlation to fish growth, but this only occurred up to 50% protein feed, while fish growth would decrease if the feed protein content was >50%. This is probably because *C. ignobilis* is only able to absorb feed protein optimally by ≤ 50%. Similar results occurred in *Oreochromis niloticus*, which was given feed treatment with 17%, 30%, and 35% protein, which showed that the optimum growth results occurred in 30% protein feed treatment, but the 35% protein feed in *O. niloticus* had no significant effect on fish growth²⁴. Furthermore, another study by Yang *et al.*²⁵ revealed that *Bidyanus bidyanus*, which was given feed treatment with protein levels of 13%, 19%, 25%, 31%, 37%, 43%, 49%, and 55%, showed that fish growth continued to increase in size from 13%–37% protein feed. However, fish growth was no longer significant in the range of 43%–55% protein feed. Other research results, also by Yang *et al.*²⁶ on *Spinibarbus hollandi*, which received protein feed of 13%, 19%, 25%, 31%, 37%, 43%, 49%, and 55%, showed that fish growth continued to increase from 13%–31% protein feed, but fish were no longer grow

significantly in the 37%–55% protein feed. This review shows that the fish's ability to absorb feed protein differs by species and that fish have a limited ability to absorb protein in feed at certain periods and conditions.

Protein is the main macromolecular component fish need²⁷. The function of protein is prioritized for the synthesis of new proteins according to the fish's nutritional needs. At the same time, carbohydrates and lipids supply energy availability for fish²⁸. Feed protein will be hydrolyzed in the digestive tract, and only free amino acids can be absorbed by the intestine for tissue and organ purposes as body protein²⁹. However, feed with high protein content may not be used optimally by fish as in this study. Feed with 60% and 70% protein given to *C. ignobilis* actually decreased the ability to grow, the feed efficiency (FE), and fish survival. In addition, the feed treatment with 60% and 70% protein given to *C. ignobilis* also decreased the average FE and SR values but increased the FCR value. High FCR will increase feed consumption so that fish farming production costs tend to increase as well. Furthermore, the SR values in protein feed treatment of 60% and 70% only reached 68.34% and 65.00%, probably because the ability to absorb feed (FE) decreases, so that the quantity of fish feces increases and causes the fish to die due to poisoning.

The mean optimum FE value of *C. ignobilis* reached 77.755%, which resulted in the 50% protein feed treatment. This result is better than the best average FE value of *C. hippos*, which reached 19.01% in the combination treatment of 50% fish waste and 50% artificial feed³⁰. Furthermore, another study by Rostika *et al.*³¹ also produced the best average FE value for *Caranx* sp. lower than this study with a value of 30.60% in the treatment of 100% trash fish feed. The different results may be due to different test fish species and feed treatments, so that the fish's ability to absorb feed nutrients is also different. Another factor that plays a role in FE is the biological value contained in feed protein. Protein in feed with high biological value will stimulate the accumulation of body protein greater than that of low biological value^{32–34}. Protein is a nutrient required in large quantities in fish feed formulations because it needs to be given continuously with sufficient quality and quantity. Buwono³⁵ states that, biologically, the quality of artificial feed shows the nutritional value of the protein contained in the feed. Thus, the feed quality is assumed to be protein quality. The feed protein quality depends mainly on its essential amino acid content; the lower the essential amino acid content, the lower the protein quality^{36–39}.

The best SR value in this study was shown in the 50% protein feed treatment with a mean value of 86.67%. This result is lower than the studies conducted by Rostika *et al.*³¹ and Rostika *et al.*³⁰ regarding the combination of pelleted feed and trash fish to *Caranx* sp. and *C. hippos*, which obtained an SR value of 100% in each treatment. Furthermore, the mean SR value of *C. melampygus* in the study of Suprayudi *et al.*²³ is also higher than this study, with a value reaching 100% with the best treatment of 45% protein feed. Furthermore, another study by Rombenso *et al.*⁴⁰ showed that *C. latus*, *C. crysos*, and

C. hippos reared in floating net cages near the coast of Brazil by feeding sardines produced an average SR value of 90%. Therefore, further research is needed to increase the SR value of *C. ignobilis* in the future so that it can increase fish production.

The best FCR value produced in this study was in the 50% protein feed treatment with a mean value of 1.29. The lowest value was shown in the treatment of 70% protein feed (mean FCR 1.96). This result is better than the research conducted by Rombenso *et al.*⁴⁰, which showed that *C. latus*, *C. crysos*, and *C. hippos* rearing in floating net cages near the coast of Brazil by feeding them sardine produced a mean FCR value of 4.2. This study's FCR value is also better than the FCR value of other fish species, such as largemouth bass *Micropterus salmoides* (best FCR value of 1.39⁴¹), Asian stinging catfish *Heteropneustes fossilis* (best FCR value of 1.42⁴²), Thai pangas *Pangasius hypophthalmus* (best FCR value of 1.65⁴³), and relatively equivalent to the FCR of the Siberian sturgeon *Acipenser baeri* (maximum of 1.25⁴⁴) and catfish *Ictalurus punctatus* (maximum of 1.30⁴⁵).

Conclusion

The results showed that feed with different protein levels had a significant effect on the mean values of DGR, SGR, AGR, FCR, FE, and SR in *C. ignobilis*. The 50% protein feed gave the best results on *C. ignobilis* with a mean DGR value of 0.27 ± 0.01 g / day, a mean SGR of $1.72 \pm 0.03\%$ / day, a mean AGR of 0.13 ± 0.01 cm/day, a mean FCR of 1.29 ± 0.07 , FE was $77.76 \pm 4.46\%$, and SR was $86.67 \pm 5.44\%$. The treatment with the lowest value was 70% protein feed with a mean DGR value of 0.12 ± 0.01 g / day, mean SGR $0.91 \pm 0.08\%$ / day,

mean AGR 0.07 ± 0.02 cm/day, mean FCR 1.96 ± 0.15 , FE $51, 19 \pm 3.71\%$, and the average SR was $65.00 \pm 3.34\%$. Furthermore, feed treatment with increased protein content between 30%–50% has a positive correlation with the growth of *C. ignobilis*. The ability to grow fish will decrease, however, if the protein content of the feed is >50%.

Data availability

Figshare: Effect of dietary protein level on growth, food utilization, food conversion and survival rate of giant trevally (*Caranx ignobilis*). <https://doi.org/10.6084/m9.figshare.12936563.v1>⁴⁶.

This project contains the following underlying data:

- Data of daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency (FE) and survival rate (SR) of *Caranx ignobilis*. (Raw data for the daily growth rate (DGR), specific growth rate (SGR), absolute growth rate (AGR), feed conversion ratio (FCR), feed efficiency (FE) and survival rate (SR) of all fish examined in this study.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Mohammad Bodrul Munir

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The MS "Effect of dietary protein level on growth, food utilization, food conversion and survival rate of giant trevally (*Caranx ignobilis*)" is not written well. For example, writing style of the background in the abstract was found to have grammatical errors. The methods in this section are expressed as future tense.

There was no feed proximate analysis found; however it is necessary to validate the desired protein percentage.

How many biological and technical replicates did you take?

Superscripts in Table 2 indicated the significance differences among the rows; but the researchers did not express this, therefore it is quite difficult to understand. Please revise it.

In the Table 2, the results of DGR in A and B were not significantly different. It should be same. Please check it. Standard errors of the results are confusing. (how is it possible 0.01, 0.02, etc?)

Expression of superscripts in Table 3 have the same problem as Table 2, please re-write. I felt confused the FCR data. Was there any relation of 3% body weight feed provided to fish? How did you calculate this 3% body weight?

Overall not at the standard for indexing using the present format.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Fish Nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 05 July 2021

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Wing Yin Mo

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1. In the abstract, please revise the Methods from future tense to past tense, as the authors have already finished the experiment.

2. Professional editing is needed to improve the overall quality of the manuscript.

3. In the introduction, I think it will be more meaningful if the following information is provided:

- Production volume of the species, exported vs. domestic consumption.
- Natural diet composition: Is the fish carnivorous fish? What are the natural preys?

4. One of the major issues of the manuscript is the feed formulation (Methods). More explanation should be provided to polish the manuscript.

- How did the authors produce the feed? Home-made or produced by manufacturers?
- Where did the authors get the ingredients? Home-made or purchased?
- Do the fish feed pellet float or sink? Does the diet fit the preference of fish?
- The design seems like the authors were testing the suitability of blood meal (I prefer “meal” instead of “flour”) rather than testing the effects of protein levels. I did a quick check. There are many studies to replace fishmeal with blood meal. For omnivorous fish, 50% replacement is suggested (Kirimi *et al.* (2016¹)). For carnivorous species, such as Murray cod, partial replacement is possible (Abery *et al.* (2002²)). Would it be true that high levels of blood meal affect growth of fish?
- There are too many variables in the diets. To my best knowledge, high levels of carbohydrate is harmful to carnivorous fish. The author might check the paper published by Stone *et al.* (2003³), for more information about the effects of carbohydrates on different fish species. Thus, it would be possible that fed with Diet A and B resulted in inferior growth is related to the high levels of carbohydrate.
- The diets were tested using juveniles and that should be reflected on the title.

5. The authors suggested fish mortality of groups D and E were related to feces accumulation and poisoning. However, the authors didn't mention the depth of the experimental pond. In the pond used in the experiment, the authors suggested nets were used. Could the fish feed accumulate inside the cage and kill the fish because of that? Did the fish show any sign of intoxication? Also, is that pond equipped with any aerators? Is there any water treatment facility?

6. Amino acids and proximate compositions of the diets: The authors calculated the protein content of fish feeds. Did the authors measure the exact protein concentration? Would it be possible that the high level of blood meal resulted in inferior growth, because of insufficient amino acid(s)? In addition to protein, other proximate compositions i.e. lipid, ash, moisture and carbohydrate contents should also be measured and presented.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Aquaculture, environmental science

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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