

Piloting the co-culture of *Gracilaria verrucosa* seaweed and *Penaeus monodon* in extensive pond systems in Java, Indonesia

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Abstract

With an increasing need for food for the growing global human population, more food will have to be produced sustainably with a lower impact on the environment while still being economically profitable. We studied the effect of combining seaweed cultivation with extensive shrimp farming on production, gross profit and nutrient concentrations. Pond experiments were conducted using different combinations of the agaroid seaweed *Gracilaria verrucosa*, and the Giant tiger prawn, *Penaeus monodon*, without supplemental feeding. Combined cultivation showed 18% higher seaweed production during the first cycle and 27% higher production during the second. Shrimp production was 53.8% higher in combined cultivation than in single-species cultivation. Seaweed quality in terms of agar content and gel strength did not differ between treatments. Gross profit of combined cultivation was, respectively, 156% and 318% higher compared to single-species seaweed and shrimp cultivation. As no feed was used, environmental impact was reduced using this method. Seaweed culture decreases nutrient loading in the water and pond sediment when cultured either solely or in combination with shrimp and lowered bacterial counts. Co-cultivation therefore improves the income whilst reducing the environmental impact.

Introduction

The increase in the global human population has an inevitable impact on the need for food as well as on the need for more-sustainable livelihood options. While greater awareness is rapidly being gained of the impact of fishing and the risks of overfishing as a source of seafood, the demand for more sustainable seafood sourcing is increasing. Concomitantly, interest and focus has greatly shifted towards aquaculture in which global production has increased by more than 500% during the past three decades (FAO 2020). In comparison, capture fisheries only increased by 14% during the same period (1990–2018) globally and are often exceeding their sustainable limits for the species in question (Zhou et al., 2015, Link & Watson, 2019).

The Indonesian archipelago has a combined coastline of over 81,000km, ensuring a large aquaculture potential which in many areas is already being utilized. Nevertheless, only part of this potential is currently in use for aquaculture with estimates ranging from 22.5–40% of the brackish water area and between 0.01% and 2.32% of the coastal marine areas (KKP 2017, Amelia et al., 2021). Notwithstanding the underused potential, Indonesia is one of the largest exporting countries of cultivated shrimp globally (Hendriksson et al., 2019). Even so, Indonesia is being outcompeted by other countries like Thailand, China and Vietnam because of lower business efficiency, lower market penetration and quality, in part due to sanitation issues (Widowati et al., 2018). A key problem in lower vitality and competitiveness of the industry is the intensification of the cultivation practices, which leads to poor water quality as result of accumulation of unused feed and faeces from the shrimp. This in turn results in higher vulnerability to epizootic disease outbreaks and more frequent shrimp mortality (Izzati, 2011, Jescovitch et al., 2017).

Two key shrimp species need to be mentioned: the Pacific white shrimp (*Litopenaeus vannamei*) which accounts for about 70% of the national shrimp production, and the Giant tiger prawn (*P. monodon*) which accounts for about 19% (Hargiyato and Sumiono, 2013, Anshary et al., 2017, Wiradana et al., 2020). Cultivation of both species takes place in ponds with additional feeding. Post larvae shrimp are placed in the ponds and left to grow out to marketable size. The time needed for this process differs substantially between the two species with approximately eight weeks for *L. vannamei*, and 16 weeks for *P. monodon*, respectively. Even though the latter species has a higher market value, its longer maturation time and higher vulnerability to disease makes it the less preferred in culture (Widowati et al., 2021a). To cultivate *P. monodon*, post larvae (PL) are released in the ponds with a maximum density up to 25 ind·m⁻². In this life stage the shrimp are benthic feeders feeding on detritus and are capable of surviving salinity changes down to 2‰ (FAO, 2021).

As ponds used for intensive aquaculture typically deteriorate rapidly and become unusable (Primavera et al. 2014), the current government strategy to expand coastal aquaculture is to abandon ponds once unproductive and expand into vital mangrove areas, thereby destroying the mangroves. It is predicted by Ilman et al. (2016) that brackish-water aquaculture will continue to be the main driver of mangrove destruction in Indonesia for the next two decades. This comes at a high cost to society due to the loss of critical natural capital (Henriksson et al., 2019). Mangrove systems have been shown to represent a much higher total economic value than coastal aquaculture systems for which they are destroyed. For instance, mangrove ecosystem input to coastal fisheries may vary from ~ 3500 US\$ ha⁻¹·yr⁻¹ in Australia (Taylor et al. 2018), 7,002 US\$ of shrimp ha⁻¹·yr⁻¹ in Gujarat, India (Das 2017), an average of 13,223 US\$ ha⁻¹·yr⁻¹ in several countries considered by Ronnback (1999), and up to as high as 37,000 US\$ ha⁻¹·yr⁻¹ in Mexico (Aburto-Oropeza et al., 2008). The economic value of coastal protection by mangroves can range between 1,586 US\$ ha⁻¹·yr⁻¹ in Kenya (Kairo et al., 2009) and 5,812 US\$ ha⁻¹·yr⁻¹ in the Philippines (Menéndez et al. 2018). These numbers should be added up to calculate the economic value of these systems. Furthermore, the Net Present Value (NPV) of mangroves has been calculated to be between 4 and 8 times that of intensive shrimp farms in India (Khor 1995) and Sri Lanka (Gunawardena & Rowan, 2005), while Total Economic Value (TEV) for Sulawesi, Indonesia, has been calculated at 36,000 US\$ ha⁻¹·yr⁻¹ (Mangkai et al., 2012) and for a case in India, at 57,000 US\$ ha⁻¹·yr⁻¹ (DebRoy, 2012). This not only means that there is a high socio-economic priority to protect the remaining mangroves but also a high premium on restoring the productivity of coastal ponds through remediation so that pond productivity can be restored and the drive to continue destroying mangroves can be reduced. Based on cases from around the world, Van Wesenbeeck et al. (2015) illustrate how mangrove destruction for aquaculture expansion induces erosion of tropical coastlines, and ultimately results in poverty, socio-economic upheaval and forced displacement of coastal communities due to loss of livelihoods (Abidin et al., 2013; Joseph et al., 2013).

The aim of this work was to study the potential of seaweed culture to help remediate and restore shrimp pond productivity in largely dysfunctional coastal shrimp ponds and reduce the deterioration of coastal zones. Co-cultivation of plant and animal crops has been proven to enhance productivity thanks to circularity (e.g. Bashir et al., 2020, Fierro-Sañudo et al., 2020, Hargrave et al., 2022). Seaweed is not only a valuable crop in itself (Gentry et al., 2020), but also can absorb excess nutrients to potentially give better water quality, healthier shrimp and higher shrimp production (Kang et al., 2020). In this way it may be utilized to help restore ecosystem health by lowering impacts on the natural system and improving the livelihood of the coastal communities. Seaweeds are efficient at absorbing and using surplus nutrients, resulting in lower farm expenses as well as lower eutrophication for a more integrated and circular production system while also diversifying farm production (Widowati et al., 2019, Widowati et al., 2021a). As productivity of shrimp farming has been

declining, more and more farmers have been transitioning to seaweed cultivation which today has become a major activity in Indonesia's coastal areas (KKP, 2017). This transition has been enhanced by the ambition of the Indonesian government to become the world leading producer of agar-agar and carrageenan products (Saleh and Sebastian, 2020).

Both agar-agar and carrageenan, are hydrocolloids which are extensively used as binding agents in both food and non-food products. About 99% of the cultivated seaweed in Indonesia is already destined for this industry and makes it the key market for seaweed producers (Rimmer et al., 2021). The main seaweed species are the red algae *Kappaphycus alvarezii*, *Eucheima denticulatum* and *Gracilaria spp.* Species of latter genus grow in temperate to tropical marine environments and are prized because of their fast growth and food-grade quality agar. Therefore, *Gracilaria* culture has been pinpointed as a priority for further development (Lüning and Pang 2003, FAO 2014).

Our specific objective in this study was to compare and contrast pond yields for separate and mixed cultures of seaweed and shrimp. By measuring inputs, and yields, product and water quality parameters for, respectively, the monoculture of shrimp, monoculture of seaweed, and combined culture of seaweed and shrimp, we aimed to assess the potential for environmental remediation and evaluate the net effect of combined culture on pond system health and pond economic output.

Materials and Methods

Experimental design

Growth and yield of Giant tiger prawn (*P. monodon*) and seaweed (*Gracilaria verrucosa*) was measured over a 30-day cultivation period in farmer ponds of the village Kaliwlingi (Brebes district, Central Java, Indonesia). Seaweed and shrimp were cultured separately and jointly using identical stocking rates for either of the two in order to assess potential differences in income generation under the three different culture scenarios. Each scenario was executed in triplicate. In addition to total production in terms of weight yield, quality parameters for seaweed (in terms of agar content and gel strength) were also measured as these are important criteria for sale of the product. We also compared two typical seeding methods, namely the method whereby seaweeds are hung in the water column and the method whereby seaweed "seedlings" are loosely broadcast into the ponds. Even though not a current market criteria of importance for sale, we also examined food safety in terms of heavy metal content in the shrimp.

The native seaweed species *G. verrucosa* was selected for the experiment as it is already used for cultivation in the surrounding region. The species is commonly cultured in pond systems under saline and brackish conditions and is processed for the production of agar-agar after drying. The initial stocking, at a density of 100g/m², took place shortly prior to the stocking of the shrimp using the broadcast method. After 30 days the seaweed was harvested, weighed and air dried on netting hung above the ground using bamboo sticks. After harvest, the ponds were restocked, and the second "hanging method" was used whereby seaweed seedlings are attached to ropes and hang freely in the water column.

Penaeus monodon post larvae (PL) of ~ 12 weeks of age were placed in the ponds at a density of 10 per m². The PL-12 shrimps had an average body weight of 0.15 gram and were stocked at a relatively low density as no additional feed was provided.

Nine ponds were chosen based on availability and willingness of the farmers to collaborate. The ponds used, were distributed within an area of approximately 1 km² and varied in size between 2,000–4,000 m². Other important agricultural activities in the area include chicken and shallot (*Allium cepa*) farming. Both practices are known to use substantial quantities of fertilizer, pesticides, antibiotics and the excreta of chickens contain substantial quantities of phosphorus potentially affecting water quality. No information was available on the scale and impact of either of these activities, but pond nutrient parameters were monitored throughout the experiment.

After selection, the ponds were treated as follows to help standardize culture conditions. Water was drained and the top layer (~ 15cm) of pond sediment was removed by hand. Excess sediment was placed on top of the adjacent dikes surrounding the ponds, following common practice. After sediment removal, the ponds were left to dry for 7–10 days until sediment cracked (~ 20% moisture content) and became odourless (WWF Indonesia, 2014) in order to kill unwanted aquatic organisms. To further reduce unwanted organisms that could affect the experiment an additional, commonly used, treatment with an organic pesticide (Saponin) was used.

Physical parameters and nutrients of pond water and sediment

Concentrations of, organic carbon, nitrate (NO₃) and phosphate (PO₄) and water were measured *ex situ* by taking samples monthly using spectrophotometry. Additional water samples were taken to determine chlorophyll according to the method of Strickland & Parson (1968) and Total dissolved solids (TDS). Salinity (digital ATAGO® PAL-06S; 1 ppt accuracy), temperature and dissolved oxygen were determined using the Water Quality Checker (YSI® Pro 20; 0.1 °C and 0.01 ppm), turbidity using the Thermo Scientific™ Orion™ AQUAfast AQ3010 and pH by means of in situ measurements with the EZDO 7200 and its ORP sensor. All abiotic parameters were measured three times a day (morning, noon and afternoon), two days each week. Temperature and irradiance (PAR) were additionally measured continuously in all ponds using HOBO-loggers (Hobo UA 003–64 Onset, using one logger per pond attached 30 cm below the water surface on a bamboo pole. In addition, one logger was placed above the pond to measure air temperature and irradiance for reference. Logger measurements were taken once every ten minutes. At the end of the experiment three sediment samples per pond were taken to determine CNP-composition and presence of heavy metals (arsenic, lead, mercury and cadmium) using Wavelength Dispersive X-ray Fluorescence (WDXRF) with a Rigaku Supermini200. All samples were analysed in the Diponegoro University, integrated laboratory facilities. Level of detection (LOD) was for all heavy metals < 0.008mg/g .

Calculation of biological and economic yield

Seaweed was sampled prior to the (re)stocking moments and at harvest to assess dry matter content and the total yield. Shrimp were sampled prior to the stocking and at harvest. Growth measurements were done by measuring 10 randomly selected individuals every second week. Biomass was measured to determine the specific growth rate (SGR) in $\% \cdot \text{day}^{-1}$ using the formula given below, in which d = day, T = number of days of cultivation, W_0 = initial wet weight, W_t = wet weight at harvest:

$$SGR(\% \cdot \text{day}^{-1}) = \frac{\ln(W_t) - \ln(W_0)}{T} \cdot 100$$

The total harvest was sold by the farmers and prices obtained were used to assess gross income. Production costs were then subtracted to determine the net income. The calculation of individual and synergistic contributions of shrimp and seaweed to total pond revenues were done following Alam et al. (2021):

$$\frac{\text{varvec{C}} \cdot \text{varvec{o}} \cdot \text{varvec{n}} \cdot \text{varvec{t}} \cdot \text{varvec{r}} \cdot \text{varvec{i}} \cdot \text{varvec{b}} \cdot \text{varvec{u}} \cdot \text{varvec{t}} \cdot \text{varvec{i}} \cdot \text{varvec{o}} \cdot \text{varvec{n}} \cdot \text{varvec{o}} \cdot \text{varvec{f}} \cdot \text{varvec{s}} \cdot \text{v}}{\text{varvec{C}} \cdot \text{varvec{o}} \cdot \text{varvec{n}} \cdot \text{varvec{t}} \cdot \text{varvec{r}} \cdot \text{varvec{i}} \cdot \text{varvec{b}} \cdot \text{varvec{u}} \cdot \text{varvec{t}} \cdot \text{varvec{i}} \cdot \text{varvec{o}} \cdot \text{varvec{n}} \cdot \text{varvec{o}} \cdot \text{varvec{f}} \cdot \text{varvec{s}} \cdot \text{v}} \cdot \%$$

Agar content and gel strength

Agar content and gel strength were determined following Rejeki et al. (2018). This method involves the following process: After drying, 500g of seaweed was pre-treated with 5L of NaOH 6% (w/w) at 85°C for 3.5h. The mixture was washed several times with tap water and neutralized with 200 mL of acetic acid 0.5% (w/w) for 1h at room temperature. Extraction was then performed with 200 mL distilled water at 85°C for 2h and the mixture was filtered while still hot using a 100% cotton cloth. The mixture was then filtered using clean filter and allowed to gel at room temperature for 24h. After this, the gelled material was removed in order to do further analysis. Based on the air-dried weight, agar yields were calculated. To determine the gel strength the Brookfield CT3 4500 was used. Dried agar (1.5 gram) starch was then dissolved in 100 ml distilled water by magnetic stirring for 20–30 min at 90°C. Stabilization of the solution was done in a 80–90°C water bath for 15 min in order to reduce and remove irregularities (Rejeki et al., 2018). From the solution, 22ml was poured into specially designed 30ml cups; the cups were cooled (20-30min) using a freezer before being sealed-off using aluminum foil. Samples were left overnight at 28°C. The next day the samples were placed on a weighing scale and a stainless cylinder rod was used to put pressure on the samples. Pressure was applied until breaking of the gel occurred using a Rheometer. The measured weight at the point of breaking divided by the rod's cross-sectional surface (0.786cm²) was used to determine the gel strength following Bono et al. (2014).

Food safety

At the end of the experiment nine samples of shrimp per pond were analysed for bacterial contamination by the Central Java laboratory of the Ministry of Fisheries in Semarang. Determination of the total number of bacterial colonies per plate (total plate count in colony-forming units per gram, CFU/g) was done according to the Indonesian National Standard procedures (Standard Nasional Indonesia) SNI 2332.3:2015. The procedures for *Escherichia coli* determinations were according to SNI 2332.1-2015, for *Salmonella* according to SNI 01-2332.2-2006, and for *Vibrio cholerae* according to SNI 01-2332.4-2006.

Statistical analysis

Statistical analysis was done using Graph Pad Prism (V8.2.1) software. Comparison between ponds within treatments was done using Welch' one-way ANOVA (under assumption of a Gaussian distribution and no equality of SD's). Agar content was tested using the similar approach. Gel strength, bacterial presence (total plate count) and seaweed production were compared using a unpaired Welch's t-test (under assumption of a Gaussian distribution and no equality of SD's). The same test was used for carbon, nitrate and phosphate content of both the water as well as the sediment, but only samples of the start and those taken at the end of the experiment were compared. Phosphate concentrations were frequently below the level of detection, in which case the minimum detection level was set as the measured level. All samples were pooled per pond and the different ponds were treated as experimental replicates. Throughout the text, mean values for reported measurements are followed by the correspond standard deviation (SD).

Results

Water quality

Table 1 provides the p-values for comparisons of water quality parameters for the three different experimental treatments. Temperature measurements (both by hand and by continuous monitoring) did not show substantial differences between the ponds. Average temperature was $31.4 \pm 2.2^\circ\text{C}$, with a minimum of 27.0°C and a maximum temperature measured of 36.0°C . A statistically significant yet small difference between the experimental treatments was measured for salinity. The ponds with the combined culture (Shrimp + Seaweed) showed an average salinity of $24.9 \pm 2.9\text{‰}$, the ponds with only shrimp an average salinity of $28.6 \pm 3.6\text{‰}$, and ponds with only seaweed a salinity of $31.6 \pm 1.8\text{‰}$. Mean turbidity for combined, shrimp and seaweed culture, respectively, 18.89 ± 12.23 , 23.04 ± 12.20 and 17.21 ± 8.31 Nephelometric Turbidity Unit (NTU). For the Total Dissolved Solids (TDS) this was, respectively, 19.29 ± 2.20 , 21.67 ± 2.63 and 23.79 ± 1.51 ppt. Dissolved oxygen (DO) increased during the day, but no notable differences were observed between treatments. Overall average DO of the ponds was $3.67 \pm 0.59\text{ppm}$ at morning, increasing to $5.15 \pm 1.05\text{ppm}$ during mid-day and ending at $5.44 \pm 1.03\text{ppm}$ by the end of the afternoon.

Table 1
Comparison between replicates (ponds) within treatments. Significant differences were predominantly found between the ponds with solely shrimp. Turbidity differed significantly between ponds within all treatments.

P-Values	Treatments		
	Combined	Shrimp	Seaweed
TSD	0.6427	< 0.001	0.8707
Sal	0.3976	< 0.001	0.9299
pH	0.4099	0.1425	0.9999
Temp	0.9913	0.3673	0.7331
DO	0.6226	0.4162	0.1961
Turb	0.0078	< 0.001	0.0332
Chl-A	0.8886	0.4146	0.6186

Sediment and water conditions

Carbon content of the sediment increased slightly during the course of culture for the ponds with only shrimp and decreased for both combined culture and culture of only seaweed, however no significant differences between the start and the end of the experiment were found. For the water, organic carbon content showed an contrasting result as it remained equal for the ponds with only shrimp but increase for the combined culture ($p = 0.082$) and for the culture of only seaweed ($p = 0.013$). Nitrate concentrations in the pond sediment by the end of the culture cycle remained equal to those at the beginning for all treatments. In contrast, the nitrogen content of the pond waters decreased for all treatments, but only significantly for the two monocultures (shrimp: $p = 0.013$, seaweed: $p = 0.010$). Phosphate content in the sediment did decrease significantly during culture for all treatments (p -values shrimp: 0.005, seaweed 0.003 and combined: 0.003). Phosphate concentration in pond waters varied over time, but no significant decrease was found comparing the start and the end of the experiments for all treatments. Averages (per treatment, $\pm$ SD) of nitrate and phosphate concentrations are shown in Table 2.

Table 2
Nitrate and phosphate concentrations at the beginning and end of the experiment in both the water and soil. Values are averages of the ponds $\pm$ SD.

		Water				Soil			
		Treatment		Start (T0)		End (T6)		Start (T0)	
Nitrate (mg/L)	Seaweed	0,97	$\pm 0,14$	0,37	$\pm 0,05$	2,93	$\pm 0,50$	3,03	$\pm 1,69$
	Shrimp	1,29	$\pm 0,19$	0,73	$\pm 0,12$	2,54	$\pm 2,50$	2,71	$\pm 0,99$
	Seaweed + Shrimp	0,78	$\pm 0,07$	0,47	$\pm 0,38$	2,73	$\pm 1,88$	2,86	$\pm 0,76$
Phosphate (mg/L)	Seaweed	0,03	$\pm 0,03$	0,02	$\pm 0,00$	0,32	$\pm 0,03$	0,04	$\pm 0,02$
	Shrimp	0,07	$\pm 0,05$	0,02	$\pm 0,00$	0,34	$\pm 0,05$	0,02	$\pm 0,00$
	Seaweed + Shrimp	0,04	$\pm 0,01$	0,02	$\pm 0,00$	0,32	$\pm 0,04$	0,02	$\pm 0,00$

Bacterial presence

At the end of the experiment, nine samples of shrimp per pond were analysed for the presence of pathogenic bacteria *E. coli*, *Salmonella*, and *V. cholerae*. In all samples, *E. coli* was below 3 MPN/g (MNP = Most Probable Number), and *Salmonella* and *V. cholerae* were not detected. Total plate counts of samples were comparable within ponds, but differed between ponds ranging from about 3.7 logCFU/g to 4.4 logCFU/g. Bacterial load appeared lower in ponds where shrimp combined with seaweed or only seaweed had been cultured ($4 \log \pm 3.85 \log$ CFU/g) than in ponds where only shrimp were cultured ($4.3 \log \pm 3.75 \log$ CFU/g). However, the apparent difference was not statistically significant ($p = 0.145$).

Seaweed Quality

Seaweed agar content increased significantly from beginning to the end of the culture cycle for cultures of only seaweed and for cultures of seaweed combined with shrimp during the first round of cultivation ($p = 0.02$). However, no similar significant increases were found during the second cultivation cycle ($p = 0.554$).

Gel strength did not differ between the initial value and the end of culture for any of the different culture types during the first cultivation period. The starting seaweed seedlings of the second cultivation period had a significantly higher gel strength than the first cultivation period. For the second set of cultivation experiments, gel strength did not change significantly in the seaweed monoculture, but a reduction was measured in the combined cultivation. While the reduction was not significant for the broadcast method ($p = 0.064$), it was for the hanging method ($p < 0.05$). However, no significant difference could be established in gel strength between the hanging and broadcast method.

Table 3

Treatments were in triplicates and per treatment samples were taken in triplicate. Mean of values and SD of means is reflected in the table. Mean of values and SD of means is reflected in the table, except for (*) three samples were taking from the batch used to stock. (**) The specific growth rate (SGR) for the seaweed was determined for the combined deployment methods.

Treatment	Time in days (cycle)	Gel strength		Agar content (%)		SGR
		Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	<i>n</i>	
<i>Stocking material</i>	0 (1)	20.2 $\pm$ 4.1	6	13.59 $\pm$ 0.75*	3	-
<i>Seaweed</i>	30 (1)	35.7 $\pm$ 8.1	9	21.15 $\pm$ 0.06	9	2.4 $\pm$ 0.15
<i>Seaweed and Shrimp Combined</i>	30 (1)	19.3 $\pm$ 13.1	9	21.62 $\pm$ 0.10	9	2.1 $\pm$ 0.55
<i>Restocking material</i>	0 (2)	41.3 $\pm$ 5.8	3	25.54 $\pm$ 0.16	9	-
<i>Seaweed</i>	30 (2)	43.8 $\pm$ 18.0	9	22.24 $\pm$ 0.20	9	3.2 $\pm$ 0.03
<i>Seaweed and Shrimp Combined (hanging)</i>	30 (2)	16.2 $\pm$ 9.2	9	24.70 $\pm$ 0.26	9	3.7 $\pm$ 0.20**
<i>Seaweed and Shrimp Combined (broadcast)</i>	30 (2)	16.7 $\pm$ 11.5	9	22.43 $\pm$ 0.20	9	

Production

Mean seaweed yield for the monoculture was, respectively, 1923 $\pm$ 25.2 and 1858 $\pm$ 34.8 kg·ha⁻¹ for the two cultivation periods. For the co-cultivation, seaweed yield was quite higher, respectively 2261 and 2361 kg·ha⁻¹. Analysis showed that seaweed production for both the first harvest experiment ($p < 0.01$) and the second harvest experiment ($p < 0.05$) was significantly higher when co-cultivation was used. Co-cultivation of shrimp and seaweed resulted in an increase of 18% during the first period and 27% during the second period.

Table 4

Overview of the production and generated income of the monocultures in relation to the co-cultivation. Numbers were converted to productivity per hectare in order to make them comparable. Price per kilogram seaweed (dried) was \$0.35 (IDR 5,000.-) and for the shrimp this was \$6.37 (IDR 90,000.-). Values are given in USD (\$) calculated using the conversion USD:IDR of 14,129.50.

		Seaweed	Shrimp	Combination
Seaweed Yield (kg·ha ⁻¹)	1st harvest (Mean $\pm$ SD)	1923.3 $\pm$ 25.2	-	2161.0 $\pm$ 348.0
	2nd harvest (Mean $\pm$ SD)	1858.3 $\pm$ 150.7	-	2361.0 $\pm$ 127.3
Shrimp yield (kg·ha ⁻¹)		-	171.7 $\pm$ 10.4	264.4 $\pm$ 47.6
Mean Revenue	1st harvest	\$ 680.61		\$ 800.14
	2nd harvest	\$ 657.61		\$ 835.53
	Shrimp		\$ 1,093.46	\$ 1,680.88
	<i>Subtotal</i>	\$ 1,338.22	\$ 1,093.46	\$ 3,316.54
Costs (IDR·ha ⁻¹)*	Pond preparation - labour	\$ -28.31	\$ -28.31	\$ -28.31
	Pond preparation - pump rent	\$ -21.23	\$ -21.23	\$ -21.23
	Pond prep. - consumables	\$ -7.08	\$ -7.08	\$ -7.08
	Seeding - stocking material	\$ -159.24	\$ -212.32	\$ -371.56
	Seeding - Labour	\$ -14.15	\$ -7.08	\$ -21.23
	Harvest - Labour	\$ -70.77	\$ -70.77	\$ -141.55
	Drying - Labour	\$ -14.15		\$ -14.15
	Drying - materials**	\$ -17.69		\$ -17.69
	<i>Subtotal</i>	\$ -332.64	\$ -346.79	\$ -622.81
Net Profit (IDR·ha ⁻¹)		\$1,005.58	\$ 746.66	\$ 2,693.73

Shrimp yield was higher when co-cultivation was applied (+ 53.8%). Shrimp yield was 171.7 kg·ha⁻¹ for monoculture and 263.89 kg·ha⁻¹ for the combined cultivation (Fig. 1). This is also reflected in the SGR of the shrimp which were 4.44 $\pm$ 0.06 and 4.79 $\pm$ 0.16 for respectively mono and co-cultivation which differed significantly as $p = 0.026$ (unpaired T-test). Due to the low replication of these field trials (only three replicates per system) test power was relatively low and p -values ($p = 0.072$) did not reach the critical level of 0.05 or less to prove a significant effect.

The largest differences in economic impact between the different culture types was seen in the total gross profit per hectare. Combining both seaweed and shrimp revenues, the gross profit of combined culture was respectively, 156% and 318% of the gross profit of cultivating either only seaweed or shrimp. For the combined cultivation, the contribution of seaweed and shrimp to gross pond production was respectively 49% and 51%.

Heavy metals

Heavy metal content (arsenic, lead, cadmium and mercury) were tested for in the sediment, shrimp tissue and seaweed. In none of the seaweed samples were these contaminants found to exceed the level of detection (0.008mg/g), except for lead (0.012mg/g) and cadmium (0.059 mg/g) in one sample of the seed used for the second cycle. None of the sediment samples had levels of mercury and cadmium to exceed the levels of detection.

Table 5

Heavy metal content (mg/g) in the cultivated shrimp and the sediment of the ponds. All values marked as "<0.008" are those being below the level of detect cadmium content of the sediment was all below the LOD for all samples. Averages were calculated using the LOD of 0.008mg/g.

Source	Treatment	Replicate	Arsenic (As)				Lead (Pb)				Mercury (Hg)				Cadmium	
			Sample			Mean	Sample			Mean	Sample			Mean	Sample	
			i	ii	iii		i	ii	iii		i	ii	iii		i	i
Shrimp	Combined	1	0.011	0.009	0.012	0.011	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.052	(
		2	0.019	0.021	0.019	0.020	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.050	(
		3	0.008	0.008	0.008	0.008	0.049	0.042	0.038	0.043	0.008	0.008	0.008	0.008	0.120	(
		Mean	0.013				0.020				0.008				0.080	
	Shrimp	1	0.008	0.008	0.008	0.008	0.012	0.013	0.011	0.012	0.018	0.015	0.017	0.017	0.057	(
		2	0.034	0.038	0.029	0.034	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.020	(
		3	0.008	0.008	0.008	0.008	0.024	0.021	0.028	0.024	0.008	0.008	0.008	0.008	0.025	(
		Mean	0.017				0.015				0.011				0.036	
	Soil	Combined	1	0.005	0.004	0.006	0.005	0.008	0.008	0.008	0.008				0.008	
			2	0.043	0.037	0.034	0.038	0.008	0.008	0.008	0.008					
			3	0.008	0.008	0.008	0.008	0.017	0.021	0.017	0.018					
			Mean	0.017				0.011								
		Shrimp	1	0.015	0.018	0.021	0.018	0.008	0.008	0.008	0.008				0.008	
			2	0.017	0.021	0.025	0.021	0.017	0.015	0.021	0.018					
			3	0.008	0.008	0.008	0.008	0.090	0.013	0.090	0.064					
			Mean	0.016				0.030								
		Seaweed	1	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008				0.008	
			2	0.008	0.008	0.008	0.008	0.041	0.058	0.046	0.048					
			3	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008					
			Mean	0.008				0.021								

Discussion

Shrimp farming has led to multiple negative environmental effects, either directly by the input of nutrients causing eutrophication, or indirectly by the loss of natural capital (e.g. mangrove forests) due to upscaling and expansion of farming activities. For large parts of the coast of Java this has had disastrous consequences resulting in the loss of land, infrastructure and livelihoods on a massive scale (Chaussard et al., 2013; Joseph et al., 2013, Tonneijk et al., 2015, Damastuti and De Groot, 2017). The same is the case for intensive shrimp farming but intensive shrimp farming is even more destructive because aside from ecosystem impacts from mangrove destruction, it involves heavy and costly feed and chemical inputs which further degrade the environment (Biao et al., 2004, Hossain et al., 2013). Henriksson et al. (2019) determined that feed is one of the biggest contributors to the environmental impact of aquaculture in Indonesia. Excessive feed, and the resulting faeces, are strong contributors to the eutrophication of coastal waters. By excluding or greatly restricting feed, a big step can be made in decreasing the negative impact of such practices. Our results confirm the expectation that seaweed culture decreases nutrient loading in the water and pond sediment when cultured either solely or in combination with shrimp. As it is known that water quality can greatly affect the vulnerability of shrimp for infections and disease (Izzati, 2011, Jescovitch et al., 2017, Anh et al., 2019), improved water quality due to co culture of seaweed with shrimp could also reduce shrimp vulnerability to disease. In turn, this allows the production of the more valued *P. monodon* instead of *P. vannamei* and allows the production of higher-value shrimp. This is supported by the count of bacteria present which were all below the maximum allowable levels and were reduced (not significantly), indicating a potential antibacterial effect of the seaweed, similar to the findings of Hoa et al. (2018). Shrimp growth and production can be enhance by co cultivation with seaweed as it can facilitate shrimp in multiple ways (Anh et al. 2019). As summarized by Anh et al. (2019), it can: act as a biofilter reducing the nutrient loads subsequently affecting water quality (Marinho-Soriano et al., 2009a,b; Izzati 2011, Susilowati et al., 2014, Andayani et al., 2016), provide shelter for predators (e.g. birds of fish) (Yang et al., 2015, Andayani et al., 2016) and act as a substrate and increase the liveable area in a pond (Fouroughifard et al. 2017). In addition to reducing nutrient loads and bacterial biomass, any number of these additional positive effects may have contributed to increased shrimp production in our culture trials. An indirect ecological consequence of the improvement of pond utilization

(by introducing joint culture of seaweed and shrimp) is the reduced need for expansion of the number of ponds for shrimp production. The development of ponds involves the cutting of mangrove forests and excavating them for aquaculture ponds (Drude de Lacerda et al., 2021). This clearing is still continuing today as intensive aquaculture practices have resulted in a vast number of ponds left unsuitable for continuation of such practices. Today the total surface abandoned of ponds in Indonesia has reached over 250,000ha (Amelia et al., 2021). By combining shrimp and seaweed culture our work shows how abandoned ponds may be revitalized to enhance productivity and total production without the need for further mangrove destruction. By combining seaweed and shrimp culture, water quality is improved by the seaweed and no additional input of feed is needed for the shrimp. By providing a new option for reducing the pressure on mangroves, the driving factors for mangrove deforestation may be reduced so that they may continue to fulfil other vital ecosystem roles such as carbon sequestration, the prevention of soil acidification and prevention of coastal erosion (Sidik & Lovelock 2013, Ilman et al., 2016, Henriksson et al., 2019). Our work indicates that combined extensive culture of seaweed and shrimp can provide considerable advantages over the current systems of extensive cultures of only shrimp or only seaweed. The fact that our experiments showed that this can be done in older ponds which are presumed to be written off due to lower production shows that old ponds can be revitalized and that the destruction of mangroves to make new ponds for income and livelihood can be made unnecessary.

The approach of not adding feed makes that cultivation with this stocking density can be considered as extensive instead of semi-intensive. Transitioning from intensive to extensive farming brings a strong reduction of feed input, such that nitrogen and phosphorus levels decreased. This also results in a reduction of labour needs for pond management. Reduction in labour needed for pond management increases time of the farmer to be spent on other activities by the farmer to generate additional income. These additional activities are already common practice in seaweed farming in Indonesia (Rimmer et al., 2021), whereas extensive shrimp farming still requires a substantial amount of labour.

Our gross profit analysis further shows the economic benefits of combined cultivation in extensive practices. A limited difference was found between costs associated with the two types of monocultures and costs of coculture is roughly the sum of both culture types. This is due to the fact that the majority of the costs involve the costs of the starting material of both commodities and the fact that some activities cannot be combined and are necessarily additive. Nonetheless, with an increased income generated by combined cultivation our experiments showed that coculture was more profitable than either of the common types of single culture being used today.

With a reduced input on costs and labour, a reduced negative impact and potential positive feedback on the environment makes the approach highly interesting to be implemented. However, to make the step of implementation its profitability is a crucial factor in the receptiveness of by the local farmers and communities. Our results show that extensive co-cultivation of *Gracilaria sp.* and *P. monodon* is more profitable than monoculture of either of those species. A major factor in making this approach interesting for farmers is the exclusion of the use of feed. This expense can take up 80% of the variable costs in the cultivation of *P. monodon* (Nisar et al., 2021) and is likely to increase as feed production costs are expected to increase in the upcoming years (Rubel et al., 2019). The additional reduction in pond management costs thanks to combined culture. contributes

Despite the ambition of increasing seaweed production, it should be taken into account that switching to solely producing seaweed might not be the optimal solution. By adding seaweeds to a shrimp aquaculture system, environmental impacts can be reduced and water quality could improve as it takes up excessive and regenerated nutrients, leading to higher shrimp production (Widowati et al., 2021). By combining two species of different trophic levels, in our case *P. monodon* and *Gracilaria verrucosa* which are both profitable and low maintenance, the farmer could have an increased profit and an additional source of protein, without increasing environmental deterioration (Widowati et al., 2019).

We conclude by pointing out that co-cultivation of shrimp and seaweed contributes to the reduction of negative environmental impact and can aid in improving the environmental condition in the coastal zone of central Java, Indonesia. Seaweed can act as a bioremediator as nutrients are consumed by the seaweeds as organic matter in the form of biomass was produced by the seaweed which can only come from local surroundings. Introducing seaweed in intensive shrimp farms might therefore be of interest to be investigated to act as a bioremediator in order to improve water quality, reduce nutrient loads in the effluent and improve overall shrimp production. Extensive farming furthermore contributed to the environmental impact as no organic matter (feed) was put into the system with the applied method. In addition, extensive farming can contribute to the livelihood of coastal communities as pond management (labour) is reduced resulting in more available time for the farmers to do other income generating activities. Also, the strong reduction in costs gives combined culture a high potential. The reduction of costs is largely driven by the exclusion of costly feed, and makes that farmers will less often need a loan to cover the cost of purchasing the feed. The combined production showed a significant increase of income enhancing the farmers income. The positive feedback of the participating farmers who adapted the methodology after the experiment was done attests to the successful implementation potential of our results.

Declarations

Competing Interest declaration

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Nauta, RW: Leading researcher, experimental design, data processing and writing
Lansbergen, RA: Project support, experimental set up, discussion
Ariyati, RW: Project support on site, field work, data interpretation and discussion, writing
Widowati, LL: Project support on site, field work, data interpretation and discussion, writing
Rejeki, S: Project management on site, discussion and data interpretation
Debrot, AO: Writing, data interpretation and discussion

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [author initials], upon reasonable request.

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Figures

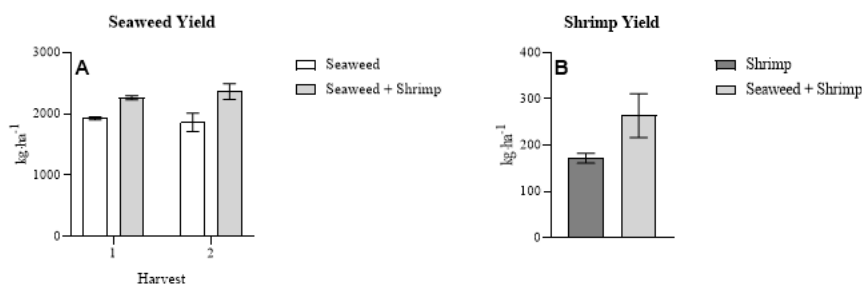


Figure 1

Yields of both commodities per harvest and per treatment, converted to kilogram per hectare. In both cultivation cycles the seaweed yield increased significantly when shrimp and seaweed were combined. For shrimp cultivation yield also increased significantly when the two commodities were cultivated jointly.