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TABLE OF CONTENTS

Animal Breeding and Production

K. Jarząbek, J. Miciński, K. Ząbek – *Effect of Dietary Supplementation with Autolyzed Saccharomyces Cerevisiae Yeast and Multi-Strain Probiotic Formulation on On Growth Performance and Longissimus Dorsi Muscle Characteristics in White-Headed Mutton Rams* 5

Biology and Forestry

A. Berroukche – *Preventive Effects of Aqueous Soybean Seed Extract on Testosterone and High-Calorie Diet-Induced Benign Prostatic Hyperplasia in a Rat* 13
D. Kubiak – *Lichens of the Paryż Nature Reserve (Ciechanów Upland, Poland)* 27

Humans and Environment

I. Effendi, I. Ismandianto, F.H. Saikim, I. Purnama, A. Rahman, N. Nurhayati, F. Yandri, A. Kurnain, R. Lovya Junakea – *Ecological Marine Ecotourism Suitability Analysis of Kelong Pancing Madu Tiga in Senggarang Besar Waters, Bintan Regency, Indonesia* 41

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EFFECT OF DIETARY SUPPLEMENTATION WITH AUTOLYZED *SACCHAROMYCES CEREVISIAE* YEAST AND MULTI-STRAIN PROBIOTIC FORMULATION ON ON GROWTH PERFORMANCE AND *LONGISSIMUS DORSI* MUSCLE CHARACTERISTICS IN WHITE-HEADED MUTTON RAMS

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K e y w o r d s: sheep, lactic acid bacteria, ultrasound method, muscle traits.

A b s t r a c t

The objective of this study was to determine the effect of dietary supplementation with autolyzed *Saccharomyces cerevisiae* and a probiotic product on the growth performance and meatiness of sheep. White-headed mutton rams aged 16 months ± 14 days (n = 18) with similar initial body weight (40.8 kg ± 1 %) were divided into three groups of six animals each: control (C) and two experimental groups (E_1, E_2). Throughout the 30-day experience period animals received a product containing *Saccharomyces cerevisiae* yeast in group II, and preparation consisting of a mixture of bacteria (*Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium animalis*) in group III. The following parameters have been determined in rams: body weight, average daily gain, growth rate, the dimensions of *musculus longissimus dorsi* sections, fat thickness over the loin-eye area. It was found that the rams administered supplements had a higher body weight, average daily gain compared to the control group, but the noted differences were not significant. The animals in experimental group also had a more favorable musculature development. Dietary supplementation with autolyzed Saccharomyces cerevisiae yeast, used alone or in combination with probiotics, had no significant effect on the growth or characteristics of the *longissimus dorsi* muscle in rams.

Introduction

Autolyzed yeast cultures are effective feed additives for ruminants. *Saccharomyces cerevisiae* is one of the most popular yeast species. It is widely used as a substrate, primarily in the form of a lyophilizate, in the

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feed industry (SHURSON 2018). Other yeast species, sourced from the dairy industry (*Kluyveromyces fragilis,* referred to as the "whey yeast") and the pulp and paper industry (*Candida utilis*, known as Torula), are also used in animal nutrition. Supplemental yeast included in livestock diets contributes to reducing feed protein costs and enhancing animal health, thus improving performance and increasing profitability in livestock production (PANG et al. 2022).

Selected yeast strains used as feed additives not only enhance the nutritional value of animal diets but also confer probiotic effects (MARKOWIAK and ŚLIŻEWSKA 2018). In young ruminants, dietary supplementation with yeast species such as *S. cerevisiae* can lead to accelerated proventriculus development, reduced incidence of enteritis, improved feed conversion, increased daily gain, and enhanced resistance to stress induced by transportation and environmental factors (ELGHANDOUR et al. 2024). Commercial yeast-derived feed additives can be divided into categories such as yeast extracts, yeast β-glucans, autolyzed yeast, and hydrolyzed yeast (TAKALLOO et al. 2020). Autolyzed yeast is a relatively novel feed material. Autolysis is a degradation process that involves the activation of intracellular enzymes responsible for solubilizing cell components withing the cell (PERRICONE et al. 2022). Autolyzed yeast is composed of the total content of yeast residues remaining after lysis. Therefore, it contains B vitamins, peptides, amino acids, and nucleotides (ADILI et al. 2020). Moreover, the autolysis of live yeast leads to the release of bioactive compounds such as polysaccharides, including mannan-oligosaccharides (MOS) and β-glucan that form yeast cell walls (BAEK et al. 2024). The composition of autolyzed yeast varies depending on the production process. Autolyzed *S. cerevisiae* yeast usually contains 55–64 % β-glucans, 35–40 % MOS, 13 % protein, 9 % lipids, and 2 % chitin (NAMTED et al. 2021). Mannan-oligosaccharides and β-glucans play an important role in the immune response (NASIRI et al. 2023). The immunoregulatory activity of β-glucans is primarily linked to its ability to stimulate or inhibit the release of cytokines by macrophages, which are involved in immune system control, or to modulate macrophage phagocytosis (PETRAVIĆ-TOMINAC et al. 2010).

The key mechanisms of action of autolyzed *S. cerevisiae* yeast in ruminants include the stabilization of ruminal microbiota by promoting the proliferation of cellulolytic bacteria, which enhances fermentation and increases the concentrations of short-chain fatty acids. The above leads to rumen pH stability and increased digestibility of neutral detergent fiber and crude protein (GIOTTO et al. 2022, TAKIYA et al. 2024). Due to the presence of other valuable components, such as bioactive metabolites and B vitamins, autolyzed yeast can support resistance mechanisms and counteract oxidative stress in animals. KNOLLINGER et al. (2022) demonstrated that autolyzed yeast

supplementation did not negatively impact nutrient digestibility, which confirms that it can be safely used in intensive cattle production. A positive effect of autolyzed yeast on the immune response of dairy cows exposed to heat stress during the transition period and early lactation was reported by NASIRI et al. (2023) and ADILI et al. (2020), respectively.

The existing literature provides limited information on the dietary use of autolyzed yeast, and even less on its combinations with probiotics. Therefore, the aim of the present study was to determine the effect of dietary supplementation with autolyzed *S. cerevisiae* and a probiotic product composed of *Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Bifidobacterium animalis* strains on the growth performance and characteristics of the *longissimus dorsi* muscle in White-headed Mutton rams.

Materials and Methods

The study was conducted in the city of Olsztyn (Region of Warmia and Mazury, Poland), located at 53°46' North latitude, 20°30' East longitude, and an altitude of 128 m. The animals were housed in a free-stall sheepfold, on polypropylene slatted floors, under standard conditions, in accordance with Directive 98/58/EC and the principles of the European Convention for the Protection of Animals. All procedures related to the animals used in this study were approved by the Local Ethics Committee for Animal Experiments in Olsztyn (Decision No. 35/2025).

The experiment was performed on 18 white-headed mutton sheep. Rams aged 16 months ± 14 days were divided into three groups of six animals each (control – C and experimental – E_1, E_2) with a similar body weight of 40.8 kg ± 1 %. In each group, rams were administered supplements in the form of an aqueous solution (80 ml) *per os* for 30 days. Group E_1 animals received a product containing autolyzed *Saccharomyces cerevisiae* yeast at 0.2 g/kg body weight. In group E_2, in addition to the yeast, a selected preparation consisting of a mixture of bacteria (*Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium animalis*) was administered at 0.03 g/kg body weight. Control group sheep received an equivalent volume of water as a placebo using the same oral administration procedure (placebo--controlled supplementation), to replicate the procedures associated with supplement administration in the experimental groups.

In control and experimental groups, animals were administered identical diets: meadow hay at 1 kg/animal/day, pre-wilted grass and legumes at 2.5 kg/animal/day, oat straw at 0.5 kg/animal/day, and oat grain at 0.3 kg/animal/day, to achieve equal feeding levels. Uniform feeding

standards were applied in in control and experimental groups, in line with the nutrient requirements of rams (OSIKOWSKI et al. 1998).

The following meat performance traits were analyzed: body weight [kg] at the beginning of the study (day 1) and on days 15 and 30 of the experiment, average daily gain [g], relative growth rate [%] at the following time intervals: days 1–15, 15–30, and 1–30 of the experiment; meatiness and fatness were determined in vivo by ultrasound examination at the beginning of the study and on days 15 and 30 of the experiment. Body weight [kg] was measured individually for each animal at the beginning of the experiment (day 1) and on days 15 and 30 using a calibrated digital mobile scale for sheep (Radwag®, model H315.4I.2000.S2). Average daily gain (ADG, g/day) was calculated for each experimental period (days 1–15, 15–30, and 1–30) as the difference between final and initial body weight divided by the number of days in the respective interval.

The relative growth rate (RGR) of lambs in selected rearing periods was determined using the below formula:

$$\text{RGR} = \frac{\text{final body weight} - \text{initial body weight}}{\frac{1}{2}\,(\text{initial body weight} + \text{final body weight})} \cdot 100\ [\%]$$

Meatiness was evaluated based on the dimensions of the *longissimus dorsi* (LD) muscle, including depth, width, and area, whereas fatness was assessed based on skin and fat thickness, and back fat thickness over the loin "eye" area. The measurements were performed after the last thoracic vertebra, i.e. between the 12^{th} and 13^{th} ribs (GERALDO et al. 2023) using the Mindray DP50 ultrasound system (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.) with a 7.5 MHz linear probe.

Statistical analyses were performed using Statistica ver. 13.3. The normality of data distribution was tested using the Kolmogorov–Smirnov and Lilliefors tests, as well as the Shapiro–Wilk W test. Further analysis was performed by comparing multiple independent samples, and the results were verified at a significance level of $p \leq 0.05$.

Results and Discussion

The body weight, RGR and ADG of rams are presented in Table 1. Animals from experimental groups E_1 and E_2 were characterized by higher final body weight and higher average daily gain than control group rams, but the noted differences were not significant.

Table 1

Body weight, average daily gain, and relative growth rate of rams

Parameter	Group					
	E_1		E_2		C	
	x̄	s	x̄	s	x̄	s
Body weight [kg]						
day 1	40.93	6.54	40.85	6.34	40.43	4.85
day 15	41.90	6.96	41.62	6.61	41.70	5.05
day 30	44.23	6.21	44.37	6.23	43.90	4.54
Average daily gain [g]						
days 1–15	64.44	44.36	51.11	41.02	84.44	45.15
days 15–30	155.56	72.50	183.33	56.37	146.67	107.83
days 1–30	110.00	31.55	117.22	30.65	115.56	47.13
Relative growth rate [%]						
days 1–15	2.25	1.34	1.83	1.37	3.08	1.49
days 15–30	5.76	3.36	6,62	2.42	5.29	4.20
days 1–30	8.01	3.08	8.44	2.43	8.37	3.93

Ultrasound scans revealed that dietary supplementation with autolyzed *S. cerevisiae* yeast, alone and in combination with a probiotic product, had no effect on the dimensions of the LD muscle of rams (Table 2). Back fat thickness over the loin "eye" area was similar in all groups.

Table 2

Ultrasound scanning of the *longissimus dorsi* muscle of rams

Parameter	Days of age	Group					
		E_1		E_2		C	
		x̄	s	x̄	s	x̄	s
Depth [cm]	1	1.91	0.11	1.89	0.17	1.91	0.28
	15	1.94	0.26	1.92	0.15	1.95	0.32
	30	1.99	0.24	2.03	0.23	2.09	0.37
Width [cm]	1	5.04	0.59	5.12	0.31	5.02	0.45
	15	5.41	0.43	5.33	0.30	5.59	0.39
	30	5.42	0.27	5.40	0.50	5.60	0.22
Area [cm^2]	1	6.76	1.10	7.07	1.04	7.20	1.27
	15	7.82	1.27	7.61	0.81	8.01	1.37
	30	8.20	1.21	8.01	1.01	8.59	1.43
Fat thickness over "eye" loin [cm]	1	0.16	0.02	0.26	0.22	0.17	0,01
	15	0.20	0.07	0.23	0.04	0.18	0.05
	30	0.21	0.06	0.23	0.05	0.20	0.02

Many studies have shown that supplemental autolyzed yeast does not increase the body weight of the analyzed animals. This could be due to the too low inclusion levels of the supplement in their diets, which likely reduced its prebiotic effects in the rumen. SCHUH et al. (2025) also found that dietary supplementation with autolyzed *S. cerevisiae* yeast at 5 g/animal/day had

no significant effect on the body weight and average daily gain of lambs. A lack of effect of yeast supplementation also reported by KNOLLINGER et al. (2022). In this the work autolyzed yeast added to diets for Holstein cows at 15 g, 30 g, and 45 g per day had no influence on the body weight of animals. In a study by PONTAROLO et al. (2021), experimental beef steers that received autolyzed yeast in the diet at 4 g and 7 g/animal/day were characterized by greater daily body weight gain and better feed conversion in the initial feedlot phase, compared with control group animals, but this effect was not observed in the following phases.

BARDUCCI et al. (2024) examined monogastric animals, namely weaned piglets fed autolyzed yeast and β-glucans as a blood plasma alternative in diets and found that daily weight gain was not affected by the supplement, whereas survival rates and feed efficiency were higher in experimental groups. Similar observations were made by NAMTED et al. (2021). In their study, the body weight gain of piglets in control and experimental groups was not differentiated by 1.0 % autolyzed yeast supplementation. In turn, autolyzed *S. cerevisiae* yeast improved the growth performance of poultry, including body weight gain and the feed conversion ratio (PERRICONE et al. 2022). A beneficial influence of supplemental yeast was observed in broilers (MUTHUSAMY et al. 2011) and Japanese quail (KORDPOUR and SALARI 2023). MUTHUSAMY et al. (2011) demonstrated that hydrolyzed yeast supplementation improved the body weight of broilers at 21 and 42 days of age, compared with the control group. In turn, live weight gain was not altered by the diet until day 21, but it increased on days 22–42 in supplemented birds.

Conclusions

It can be concluded that dietary supplementation with autolyzed *S. cerevisiae* yeast culture, alone and in combination with a probiotic product, had no effect on the dimensions and fat content of the LD muscle or on performance parameters such as the body weight, average daily gain, and growth rate of rams housed in a conventional system.

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PREVENTIVE EFFECTS OF AQUEOUS SOYBEAN SEED EXTRACT ON TESTOSTERONE AND HIGH-CALORIE DIET-INDUCED BENIGN PROSTATIC HYPERPLASIA IN A RAT

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K e y w o r d s: benign prostatic hyperplasia; soybean extract; testosterone; antioxidant activity; high-calorie diet.

A b s t r a c t

Benign prostatic hyperplasia (BPH) is a common disorder in aging men characterized by non-malignant prostate enlargement and lower urinary tract symptoms. Elevated testosterone and high-calorie diets are major risk factors. This study investigated the protective effect of an aqueous soybean seed extract, rich in isoflavones and polyphenols, in a rat model of BPH induced by testosterone administration and high-calorie feeding. The extract exhibited antioxidant activity (4 % DPPH radical scavenging) and contained 33.3 mg GAE/g total polyphenols. Male Wistar rats were divided into four groups: normal control, BPH-induced (testosterone 0.02 mL/week + high-calorie diet for 90 days), BPH-induced + soybean extract (10 g/L in drinking water), and extract-only. The BPH-induced group showed significant increases in body weight (+123 g), serum triglycerides, PSA (0.41 ng/mL), and marked prostatic hypertrophy with widened acini. Co-administration of soybean extract significantly attenuated these changes: body weight gain reduced to +90 g, triglycerides dropped to 0.70 g/L, PSA returned to control levels, and histological examination revealed narrower acini and preserved prostate architecture. These results demonstrate that soybean seed extract effectively prevents testosterone- and high-calorie diet-induced BPH in rats, likely through its antioxidant, lipid-lowering, and hormonal modulatory properties. The findings support the potential of soybean-derived compounds as a natural preventive strategy against BPH.

Introduction

Benign prostatic hyperplasia (BPH) represents one of the most common urological disorders in men over 50 years of age, affecting approximately 50 % of this demographic and rising to 80 % in those over 80 (ROEHRBORN 2005). Clinically, BPH manifests as an enlargement of the prostate gland,

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leading to compression of the urethra and subsequent lower urinary tract symptoms (LUTS), including frequent urination, nocturia, and urinary retention (SPEAKMAN et al. 2004). If untreated, BPH can progress to complications such as acute urinary retention, urinary tract infections, or even renal impairment. The economic burden is substantial, with global healthcare costs exceeding billions annually due to medical consultations, pharmacotherapy, and surgical interventions like transurethral resection of the prostate (TURP). The etiology of BPH is multifactorial, involving hormonal, inflammatory, and metabolic pathways. Androgens, particularly testosterone and its metabolite dihydrotestosterone (DHT), play a pivotal role by binding to androgen receptors in prostate stromal and epithelial cells, promoting cellular proliferation. DHT is formed via the enzyme 5α-reductase, and inhibitors like finasteride target this pathway but often come with side effects such as sexual dysfunction (BERGES et al. 1995). Additionally, metabolic syndrome-exacerbated by high-calorie diets rich in fats and sugars--contributes to BPH through insulin resistance, oxidative stress, and chronic inflammation (KRISTAL and SCHENK 2006). Obesity-induced adipokines and cytokines further amplify prostate growth, creating a vicious cycle (AMARAL et al. 2015). In this context, natural interventions have gained traction as safer alternatives or adjuncts to conventional therapy. Soybeans (*Glycine max*), a legume staple in Asian cuisines, are abundant in bioactive compounds including isoflavones (genistein, daidzein, glycitein), polyphenols, and saponins (MESSINA 2010). Isoflavones act as phytoestrogens, weakly binding to estrogen receptors and potentially inhibiting 5α-reductase, thus reducing DHT levels (AWAD and FINK 2000). Epidemiological studies reveal that Asian men, with higher soy intake (20–30 g/day), exhibit a 50–70 % lower incidence of BPH and prostate cancer compared to Western populations (NAGATA 2000, HAMILTON-REEVES et al. 2010). Animal models support this for instance, anthocyanins from black soybeans reduced prostate weight: in testosterone-induced BPH rats by promoting apoptosis (JANG et al. 2010). Similarly, Seoritae (black soybean) extract decreased oxidative stress and 5α-reductase activity in rat models (REN et al. 2014). Despite these insights, gaps remain in understanding soy's effects on combined hormonal and dietary BPH induction. Most studies focus on isolated isoflavones, overlooking whole extracts' synergistic effects (LEPHART 2019). This study addresses this by investigating an aqueous soybean seed extract from Algerian cultivars on testosterone- and high-calorie diet-induced BPH in rats. We hypothesize that the extract's polyphenols and isoflavones will mitigate hypertrophy via antioxidant, anti-inflammatory, and hormonal modulation pathways. By integrating biochemical, histological, and phytochemical analyses, this work aims to provide evidence for soy as a preventive nutraceutical, potentially informing dietary guidelines for at-risk populations.

Materials and Methods

Plant material

Soybean seeds (*Glycine max* L., *Fabaceae* family) were sourced from the Béchar region in southwestern Algeria, sown in late August and harvested in November. Seeds were air-dried at room temperature away from direct light, ground into fine powder using an electric grinder, and stored in airtight containers protected from light and humidity to preserve bioactive compounds. This sourcing aligns with sustainable agricultural practices in arid regions, ensuring minimal environmental impact.

Preparation of the aqueous extract

Ten grams of soybean powder were macerated in 150 mL distilled water at room temperature with continuous stirring for 24 hours. The mixture was filtered through Whatman No. 1 paper, centrifuged at 3500 rpm for 5 minutes, and the supernatant concentrated via rotary evaporation at 100 °C to yield a brown dry extract. The residue was oven-dried at 50 °C. Extraction yield was calculated as

$$R\ [\%] = (M / M_0) \cdot 100,$$

where:

M – the mass of dried extract

M_0 – the initial plant material mass.

This method was chosen for its eco-friendliness and relevance to traditional aqueous preparations, yielding 8.23 %, comparable to literature values of 5–10 % for aqueous soy extractions (GÖRS et al. 2020).

Phytochemical analyses

Total polyphenol content

Polyphenols were quantified using the Folin-Ciocalteu method (SERAIRI-BEJI et al. 2017). Briefly, 100 µl extract (various concentrations) was mixed with 0.5 mL distilled water and 0.125 mL Folin-Ciocalteu reagent. After 6 minutes, 1.25 mL 7 % sodium carbonate was added, volume adjusted to 3 mL, and incubated in the dark for 90 minutes. Absorbance at 760 nm was measured against a gallic acid standard curve, expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g). Triplicates ensured reliability.

Antioxidant activity

DPPH radical scavenging was assessed per ATHAMENA et al. (2010). Fifty µl extract (methanolic dilutions) was added to 1.95 mL 0.025 g/L DPPH solution. After 30 minutes dark incubation, absorbance at 515 nm was read, with ascorbic acid as positive control.

$$\text{Inhibition [\%]} = [(A_control - A_sample) / A_control] \cdot 100.$$

This assay evaluates free radical neutralization, crucial for countering oxidative stress in BPH.

In vivo study

Animal handling and grouping

Forty male Wistar rats (8 weeks old, 64–84 g) were acclimatized under standard conditions (25 °C, 12/12 h light/dark cycle, standard pellet diet, ad libitum water). Rats were randomized into four groups (n = 10/group):

– group 1: Normal control (standard diet + water) ;

– group 2: BPH-induced (intraperitoneal testosterone 0.02 mL/week [concentration: 125 mg/mL in olive oil] + high-calorie diet: 50 mL milk + 10 g cheese + 10 g chocolate daily);

– group 3: BPH-induced + oral soybean extract (2 mL/day, 10 g/L);

– group 4: soybean extract only (2 mL/day, 10 g/L).

Treatment lasted 90 days, with body weight and blood parameters monitored every 15 days. Protocols complied with the European Directive 2010/63/EU and Institutional Animal Care guidelines (RUSSELL and BURCH 1959).

Biochemical analyses

Fasted blood samples (12 h) were collected from the retro-orbital sinus into heparinized tubes, centrifuged (3000 rpm, 15 min), and serum stored at -20 °C. Glucose was measured via glucometer (Vital Check). Urea, creatinine, cholesterol, triglycerides, and transaminases used SPINREACT kits (KAPLAN et al. 1984). PSA was quantified by VIDAS ELISA (BioMérieux, France). Hematological parameters (e.g., lymphocytes) were analyzed using standard counters.

Histological examination

Post-anesthesia (chloroform), rats were sacrificed, prostates excised, fixed in 10 % formalin, dehydrated, paraffin-embedded, sectioned (5 µm), stained with hematoxylin-eosin, and examined microscopically (HOULOD 1984).

Statistical analysis

Data expressed as mean ± SEM. One-way ANOVA followed by Tukey's test and Student's *t*-test ($p < 0.05$ significance) using SigmaPlot v11.

Results

Phytochemical characterization of the aqueous soybean extract

The aqueous extract obtained by maceration of soybean powder exhibited a brown coloration and complete solubility in water, with an extraction yield of 8.23 %. Qualitative phytochemical screening confirmed the presence of several classes of secondary metabolites, including flavonoids, tannins, and saponins, based on characteristic precipitation and color reactions with specific reagents.

The extract demonstrated moderate in vitro antioxidant activity in the DPPH radical-scavenging assay, showing 4 % inhibition at the tested concentration, with a calculated IC_{50} value of 91.97 mg/mL (Figure 1). Quantitative analysis revealed a total polyphenol content of 33.39 ± 01.50 µg GAE/g of dry extract (Figure 2).

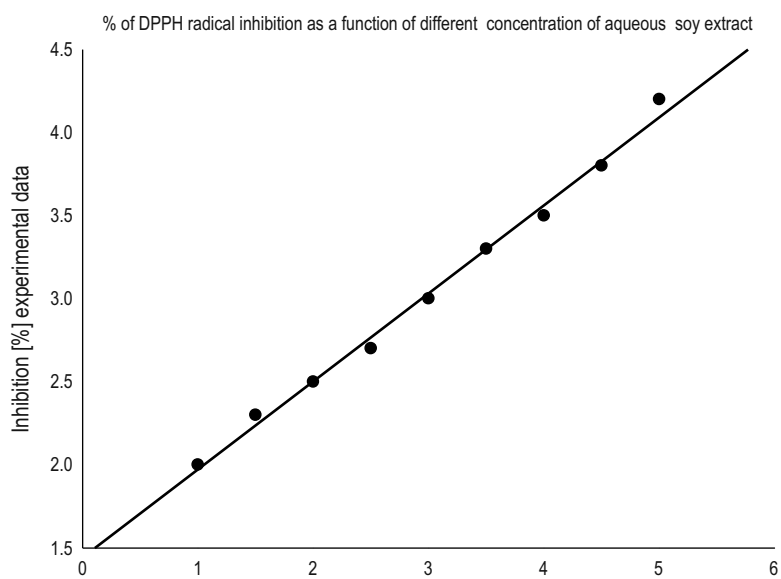


Fig. 1. Antioxidant activity of aqueous extract of soybean seed powder

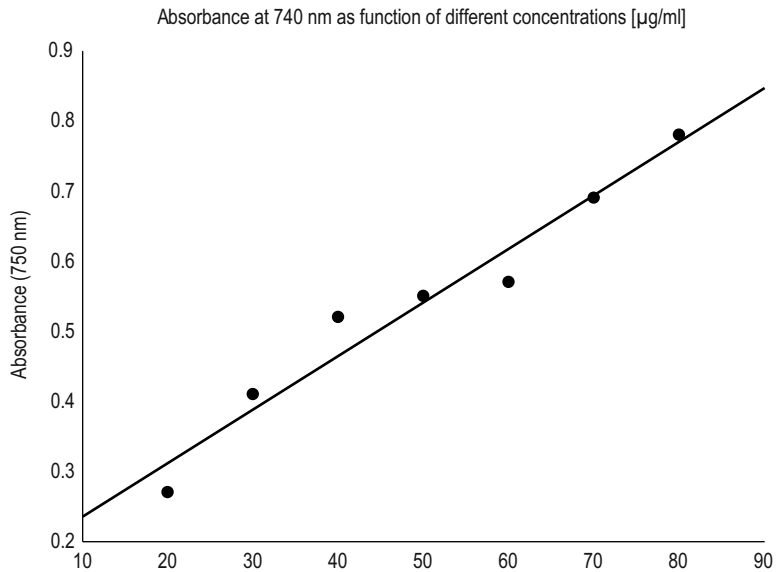


Fig. 2. Determination of total polyphenol levels

Adverse effects following testosterone administration

During the initial 48 hours of the experiment, animals receiving intraperitoneal testosterone (groups 2 and 3) displayed transient behavioral changes, including reduced general activity, impaired locomotion, and occasional abdominal contractions (Table 1). No local inflammation at the injection site or mortality was observed in any group throughout the study period.

Table 1

Adverse effects following intraperitoneal injection of testosterone in male rats

Groups of rats	Group 1	Group 2	Group 3	Group 4
Abdominal contraction	-	+	+	-
Locomotion disorders	-	+	+	-
Reduction in activity	-	+	+	-
Edema of the peritoneum	-	+	-	-
Inflammation of the testosterone injection site	-	-	-	-
Mortality	-	-	-	-

Explanation: group 1 – normal controls received a standard food; group 2 – experimental controls received high-caloric diet (HCD) and intraperitoneal injection of testosterone; group 3 – rats received HCD, orally aqueous extract of soybean powder (AESP) and intraperitoneal injection of testosterone; group 4 – rats received HCD, orally aqueous extract of soybean powder (AESP); (-) means no side effects; (+) means presence of side effects

Body weight

As shown in Table 2, testosterone administration combined with a high-calorie diet (group 2) induced a significant increase in final body weight compared to the normal control group (122.91 ± 15.18 g vs. 111.79 ± 11.03 g; $*p* < 0.05$). Co-treatment with soybean extract (group 3) markedly attenuated this weight gain (90.66 ± 6.84 g), a value that did not differ significantly from that of the extract-only group (group 4, 91.50 ± 5.77 g).

Table 2

Quantitative parameters of BPH induction and soybean extract protection

Parameter	Group 1	Group 2	Group 3	Group 4
Body weight [g]	111.79 ± 11.03	122.91 ± 15.18*	90.66 ± 6.84#	91.50 ± 5.77
Prostate weight [g]	0.77 ± 0.08	1.65 ± 0.22**	1.15 ± 0.15#	0.94 ± 0.10
PSA [ng/mL]	0.35 ± 0.03	0.41 ± 0.04*	0.39 ± 0.03	0.35 ± 0.03
Triglycerides [g/L]	0.47 ± 0.03	0.81 ± 0.11**	0.70 ± 0.04#	0.60 ± 031

Explanation: mean ± SEM; $*p < 0.05$, $**p < 0.01$ vs group 1; $\#p < 0.05$ vs group 2 (ANOVA + Tukey); group 1 – control; group 2 – BPH; group 3 – BPH + soy; group 4 – soy

Serum lipid profile

The BPH-induced group (group 2) exhibited a pronounced dyslipidemia, with significant elevations in serum triglycerides (0.81 ± 0.11 g/L vs. 0.47 ± 0.03 g/L in controls; $*p* < 0.01$) – Table 2. Administration of soybean extract together with testosterone (group 3) partially reversed these alterations, reducing triglycerides to 0.70 ± 0.04 g/L ($*p* < 0.05$ vs. group 2). The extract-alone group (group 4) showed lipid values comparable to those of normal controls.

Prostate – specific antigen (PSA)

Serum PSA, a key biomarker of prostate enlargement, was significantly elevated in group 2 (0.41 ± 0.04 ng/mL) compared to the control group (0.35 ± 0.03 ng/mL; $*p* < 0.05$). This increase was prevented by soybean extract co-administration (group 3: 0.39 ± 0.03 ng/mL) and values in the extract-only group (group 4: 0.35 ± 0.03 ng/mL) remained at baseline levels (Table 2).

Hormonal parameter

Testosterone and high-calorie diet provoked a mild inflammatory response, reflected by elevated counts of polymorphonuclear cells (8.5 ± 1.2 g/L) and lymphocytes (5.6 ± 0.8 g/L) in group 2 compared to controls. Soybean extract treatment (group 4) reduced these levels to 3.9 ± 0.5 g/L and 2.8 ± 0.4 g/L, respectively (Table 2).

Macroscopic and histopathological analysis of prostate tissue

Prostate weight and dimensions were markedly increased in testosterone- and high-calorie diet-treated rats (group 2) compared to normal controls (Table 3). The prostate index (prostate-to-body weight ratio) was approximately two-fold higher in group 2 ($13.4 \cdot 10^{-3}$) than in group 1 ($6.88 \cdot 10^{-3}$), indicating significant glandular enlargement. Co-administration of soybean extract (group 3) reduced both absolute prostate weight and the prostate index relative to the BPH-induced group, while animals receiving the extract alone (group 4) exhibited values comparable to controls (Table 3).

Table 3

Macroscopic and morphological characteristics of prostate tissues taken from rats of different groups

Groups of rats	Group 1	Group 2	Group 3	Group 4
Appearance	normal, spongy	hypertrophy	less hyperplastic	normal
Shape	regular	irregular	more or less regular	regular
Color	reddish	reddish	reddish	reddish
Size (l × w in cm)	1.5 · 0.9	2.4 · 1.1	1.9 · 0.12	1.7 · 0.43
Prostatic weight [g]	0.77	1.65	1.15	0.94
Body weight [g]	111.79	122.91	90.66	91.50
Index (prostate weight/ body weight)	$6.88 \cdot 10^{-3}$	$13.4 \cdot 10^{-3}$	$12.6 \cdot 10^{-3}$	$10.2 \cdot 10^{-3}$

Histological examination (Figure 3) revealed pronounced architectural disruption in group 2. Prostate acini were markedly dilated, irregularly shaped, and often ruptured, with expanded interglandular stroma and fibromuscular tissue. In contrast, prostate sections from control rats (group 1) displayed normal, regularly sized acini and intact tissue organization.

Notably, soybean extract co-treatment (group 3) attenuated these pathological changes: acinar dilation and stromal expansion were less severe, and tissue architecture was partially preserved. Rats administered only

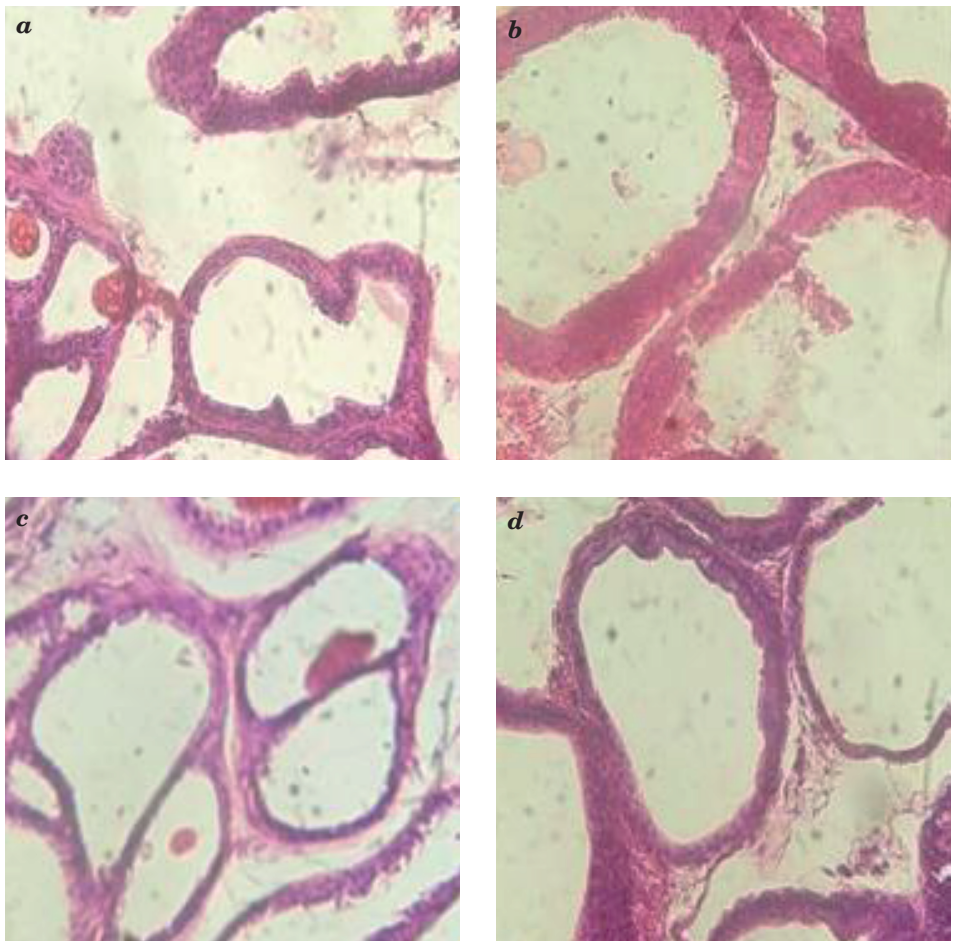


Fig. 3. Micrographs of prostate tissue samples taken from adult rats (Haematoxylin-Eosin stained histological sections observed at × 40 magnification): *a* – groupe 1 (controls); *b* – groupe 2 (HCD + testosterone); *c* – groupe 3 (HCD + testosterone + AESP); *d* – groupe 4 (HCD + AESP). Explanation: HDC – high-caloric diet; AESP – aqueous extract soybean powder

the soybean extract (group 4) showed prostate histology indistinguishable from that of the control group, confirming the absence of adverse effects from the extract itself.

Discussion

This study provides evidence that a simple aqueous extract of soybean seeds attenuates the development of benign prostatic hyperplasia in a rat model combining testosterone administration with a high-calorie diet-a

design that closely mimics two major risk factors in humans. The observed protection was multifactorial, involving improvements in prostate histology, serum biomarkers, lipid metabolism, and inflammatory markers.

The aqueous extraction yielded 8.23 % of dry material, a value consistent with other water-based preparations of soybean (GÖRS et al. 2020). The extract contained appreciable levels of polyphenols (33.3 mg GAE/g), comparable to those reported for various soybean cultivars (MARQUES et al. 2019). Although its in vitro DPPH radical-scavenging activity was modest (4 % inhibition, IC_{50} = 91.97 mg/mL), the in vivo benefits suggest that bioactive compounds such as isoflavones (genistein, daidzein) and other polyphenols act through mechanisms beyond direct antioxidant capacity, possibly involving receptor modulation and anti-inflammatory pathways (MESSINA 2010, LEPHART 2019). The whole-extract approach likely harnesses synergistic interactions among its constituents, an advantage over single-compound interventions (RUSSO et al. 2017).

Although the precise quantification of individual isoflavones was not performed in this study, the aqueous extract was prepared from whole soybean seeds known to contain high levels of genistein and daidzein, which have well-documented antiandrogenic and 5α-reductase inhibitory activities (MESSINA 2010, HWANG et al. 2021). Future studies should include HPLC analysis of isoflavone content and measurement of serum testosterone levels to further elucidate the mechanism of action

The combined testosterone and high-calorie diet trigered metabolic alterations characteristic of preclinical BPH and metabolic syndrome: increased body weight, hyperlipidemia, and elevated prostate-specific antigen (PSA). Soybean extract co-treatment significantly counteracted these changes. The reduction in body weight and normalization of triglyceride level align with the known hypolipidemic and insulin-sensitizing properties of soy isoflavones, which can activate PPAR-α/γ pathways and improve lipid homeostasis (REN et al. 2014). The decrease in PSA, a surrogate marker of androgen activity, points to interference with androgen signaling, consistent with reports that phytoestrogens compete with dihydrotestosterone (DHT) for androgen-receptor binding and inhibit 5α-reductase activity (AWAD and FINK 2000, HWANG et al. 2021).

Testosterone and diet-induced BPH was associated with elevated circulating leukocytes and lymphocytes, reflecting a low-grade inflammatory state. Soybean extract reduced these cellular markers, supporting an immunomodulatory role, possibly via inhibition of NF-κB-dependent cytokine production.

It should be noted that the animals used in this study were intact males with endogenous testosterone production. While this may have contributed to variability, it also more closely mirrors the physiological

condition in humans, in whom BPH develops in the presence of endogenous androgens. Nevertheless, future studies using castrated animals with controlled testosterone replacement could provide further insight into the direct antiandrogenic effects of soybean extract.

Most notably, histopathological analysis revealed that the extract preserved prostatic architecture: acinar dilation, stromal expansion, and glandular disorganization – hallmarks of BPH in group 2 – were markedly attenuated in treated animals. This structural protection parallels findings with black-soybean anthocyanins, which promoted apoptosis and reduced epithelial hyperplasia in testosterone-induced models (JANG et al. 2010). The mechanism may involve both antiproliferative (cell-cycle arrest) and pro-apoptotic signals, as suggested by studies showing upregulation of pro-apoptotic proteins (e.g., Bax) and downregulation of survival pathways (RUSSO et al. 2017).

Our results extend previous work on soybean isoflavones by demonstrating efficacy in a dual-factor model that incorporates dietary metabolic stress. The magnitude of protection-seen in prostate index, lipid profile, and tissue morphology – is comparable to that reported for standardized soy extracts (e.g., Seoritae) and other phytoestrogen-rich preparations (REN et al. 2014, HWANG et al. 2021). The use of a crude aqueous extract also highlights the potential of simple, food-compatible preparations for preventive nutrition. Epidemiological observations of lower BPH prevalence in populations with high soy consumption (NAGATA 2000, HAMILTON-REEVES et al. 2010) find mechanistic support in these experimental outcomes.

Certain limitation should be acknowledged as the use of a whole extract, though ecologically relevant, makes it difficult to attribute effects to specific compounds; future work should include dose-response analyses and comparisons with purified isoflavones.

Conclusion

Despite certain limitations, the present data indicate that aqueous soybean seed extract exerts a protective effect against testosterone- and diet-induced BPH in rats, likely through a combination of anti-androgenic, hypolipidemic, antioxidant, and anti-inflammatory actions. These preclinical results provide a rational basis for considering soy-based interventions in men at risk of BPH, particularly those with concomitant metabolic syndrome. Future research should focus on optimizing dosage and formulation, elucidating the underlying molecular pathways (e.g., AR signaling, apoptosis markers), and conducting well-designed clinical trials to evaluate the efficacy of soybean preparations in human BPH prevention and management.

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LICHENS OF THE PARYŻ NATURE RESERVE (CIECHANÓW UPLAND, POLAND)

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K e y w o r d s: lichenized fungi, species diversity, distribution, old-growth forests.

A b s t r a c t

This paper presents the first data on the lichen biota of the Paryż Nature Reserve, established in 2025 as part of the "100 Reserves for the 100th Anniversary of the State Forests" initiative. The study was based on field investigations conducted in 2017 in an old-growth pine-oak forest area previously designated as an in situ forest genetic conservation area. A total of 63 lichen species and one non-lichenized saprobic fungus were recorded. Two legally protected species were identified: *Chrysothrix candelaris* (strict protection) and *Hypogymnia tubulosa* (partial protection). Twelve species are included in the Polish Red List of lichens, representing the following threat categories: CR (1 species), EN (2), VU (2), LC (1), and NT (6). Three taxa, *Chrysothrix candelaris*, *Fellhanera gyrophorica*, and *Microcalicium disseminatum* are regarded as indicators of lowland old-growth forests in Poland. In addition, several species that are widespread nationally but rarely recorded in managed forests were found. Until recently, the North Mazovian Lowland remained poorly studied from a lichenological perspective, and the results presented here demonstrate that intensified surveys in this region may significantly improve knowledge of the distribution ranges and habitat requirements of rare and insufficiently known lichen species in Poland.

Introduction

Studies of lichen diversity within a given area provide not only fundamental data on the distribution of individual species and their habitat preferences, but also valuable insights into local bioecological conditions. Due to their specific biology (NASH 2008a), lichens are particularly sensitive to a wide range of anthropogenic pressures (CIEŚLIŃSKI and CZYŻEWSKA 1992, KANYUNGULU and FARKAS 2026). In addition to air pollution (BATES 2004, NASH 2008b), lichens are strongly influenced by human activities such as agriculture (LOPPI and DE DOMINICIS 1996, RUOSS 1999, PAOLI et al.

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2006, ZARABSKA-BOŻEJEWICZ 2020), infrastructure development (BROWN 1996, MOTIEJŪNAITĖ and FAŁTYNOWICZ 2005), and intensive forestry (NASCIMBENE et al. 2013, BOCH et al. 2021, HUETTMANN and YOUNG 2022). Changes in land use result in habitat fragmentation and reduced availability of suitable substrates. In forested areas, the main effects of habitat fragmentation and isolation on lichens include changes in microclimate (edge effects), reduced population size and local extinctions, disrupted dispersal, decreased genetic diversity, loss of specialist species, and ecological debt (extinction debt) (BOUDREAULT et al. 2008, SCHEIDEGGER et al. 2012, LÄTTMAN et al. 2014, PYKÄLÄ 2019, ESSEEN et al. 2022, POTENZA et al. 2022, GHEZA et al. 2024, HÄMÄLÄINEN and FAHRIG 2024). At the same time, some species or species groups are among the most reliable bioindicators of forest quality or continuity (ROSE 1976, COPPINS and COPPINS 2002, CIEŚLIŃSKI et al. 1996, CZYŻEWSKA and CIEŚLIŃSKI 2003) as they are sessile, generally slow-growing, and highly specific to particular environmental conditions and substrates (GLOOR et al. 2024). Despite the widespread application of lichens in environmental assessment, the effectiveness of this group of organisms in monitoring ecological change depends on the availability of reliable and well-documented baseline data (SEAWARD 2004, GIORDANI and BRUNIALTI 2015). Such data remain unavailable for many areas or are incomplete or outdated, which is particularly problematic in the case of nature reserves. One of the principal objectives of reserve-based conservation is the preservation of biological diversity, which cannot be achieved without prior identification and assessment of the species resources that are, by definition, subject to protection (Ustawa z 16 kwietnia 2004… Dz.U. 2004, nr 92, poz. 880).

In Poland, more than 100 new nature reserves were established in the past year under the initiative *100 Reserves for the 100th Anniversary of the State Forests*, representing an unprecedented expansion of the protected area network. The Paryż Nature Reserve is one of four reserves created under this programme in the Mazowieckie Voivodeship and the second reserve designated within the Płońsk Forest District, alongside the Pieńki Rzewińskie Nature Reserve. Consequently, the total number of nature reserves within the district has increased to four; the Dziektarzewo and Noskowo Nature Reserves were established earlier, in 1964 and 1977, respectively.

The aim of the present study is to provide the first lichenological inventory of the area currently protected as the Paryż Nature Reserve, thereby supplying baseline data essential for future monitoring and management of this newly established protected area.

Study area

The Paryż Nature Reserve was established by an ordinance of the Regional Director for Environmental Protection in Warsaw dated 31 March 2025. It is located in Płońsk County, Sochocin Municipality, approximately 1 km north of the village of Zawady B. According to the physico-geographical regionalization of Poland (SOLON et al. 2018), the reserve is located within the North Mazovian Lowland, in the western part of the Ciechanów Upland. In the ATPOL grid system (ZAJĄC 1978), used for presenting the distribution of lichens in Poland (CIEŚLIŃSKI and FAŁTYNOWICZ 1993), the area is situated in grid square Ce63.

Under the above-mentioned ordinance, forest compartments 145 a, b, c, d, f and g of the Płońsk Forest District (Paryż Forest Range), with a total area of 44.16 ha, were placed under protection. The objective of protection is "the preservation of a fragment of oak-hornbeam forests constituting a valuable element of the landscape of the Płońsk Upland". Based on its primary conservation target, the reserve was classified as a phytocoenotic reserve (subtype: forest communities) and, in terms of its main ecosystem type, as a forest reserve (subtype: lowland mixed forests) (CRFOP 2026). Within the reserve, fresh mixed forest habitats (LMŚw) predominate, supporting a pine-oak tree stand up to 184 years old, with admixtures of hornbeam, lime, elm, black alder and European aspen. From a phytosociological perspective, these communities have been classified as subcontinental oak-hornbeam forest *Tilio-Carpinetum betuli* and continental mixed coniferous forest *Querco roboris-Pinetum* (Figure 1). Small fragments of the reserve are also



Fig. 1. A fragment of the pine-oak stand in forest compartment 145b

occupied by secondary forest communities with a notable share of Scots pine (BDOL 2026). A buffer zone covering 347.76 ha has been designated around the reserve. It consists mainly of pine stands aged 48–84 years, growing on similar fresh mixed forest habitats. Prior to the establishment of the reserve, part of its present area had already been protected as an in situ conservation area (Powierzchnia Zachowawcza in situ), with the Scots pine population as the object of protection. This area was established under the Programme for the Conservation of Forest Genetic Resources and Selective Breeding of Forest Trees in Poland (MATRAS 2013) and comprised forest compartments 145b and 154c, with a total area of 20.52 ha.

Material and Methods

Field studies were conducted in April 2017 within the conservation area. A systematic walk-through survey method was applied, involving thorough searches for lichen species across the entire study area, in all potential habitats and on all available substrates. Species that could be reliably identified in the field were recorded without collecting reference specimens. In other cases, small fragments of thalli were collected for detailed taxonomic analyses under laboratory conditions. During the identification of the collected material, particularly sterile crustose lichens, standard morphological and anatomical analyses and spot tests were supplemented with thin-layer chromatography (TLC), enabling the identification of secondary metabolites of lichen mycobionts (ORANGE et al. 2001, KUBIAK and KUKWA 2011). The preserved herbarium material was deposited in the herbarium of the Department of Microbiology and Mycology, University of Warmia and Mazury in Olsztyn. Taxonomic nomenclature follows FAŁTYNOWICZ et al. (2024). In the list of recorded species, taxa are arranged alphabetically. For each taxon, the colonised substrate is given, as well as information on species status, including legal protection, threat category (according to the national Red List, CIEŚLIŃSKI et al. 2006), and bioindicator value in the assessment of lowland old-growth forests in Poland (CZYŻEWSKA and CIEŚLIŃSKI 2003).

Results and Discussion

A total of 63 lichen species and one non-lichenized saprobic fungus, *Microcalicium disseminatum*, traditionally included in lichen inventories, were recorded within the reserve (Table 1). Considering the relatively small area of the reserve and the characteristics of the surrounding forests, this

Table 1

A list of lichen species recorded in the Paryż Nature Reserve

Species	Substrates	Conservation value
1	2	3
Agonimia repleta Czarnota & Coppins	Qr	–
Amandinea punctata (Hoffm.) Coppins & Scheid.	Qr	–
Anisomeridium polypori (Ellis & Everh.) M.E. Barr	Pt, Qr	–
Arthonia radiata (Pers.) Ach.	Cb	–
Bacidina modesta (Zwackh ex Vain.) S. Ekman	Cb, Tc	–
Biatora efflorescens (Hedl.) Räsänen	Cb	VU
Buellia griseovirens (Turner & Borrer ex Sm.) Almb.	Cb	–
Chaenotheca ferruginea (Turner ex Sm.) Mig.	Qr, Tc, Ps	–
Chaenotheca furfuracea (L.) Tibell	Qr,	NT
Chaenotheca trichialis (Ach.) Th. Fr.	Bp	NT
Chaenotheca xyloxena Nádv.	wd;	VU
Chrysothrix candelaris (L.) J.R. Laundon	Qr	SP, CR, Ind.
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng	Qr	–
Cladonia coniocraea (Flörke) Spreng.	Cb, Tc, Ps, Pt, wd	–
Cladonia digitata (L.) Hoffm.	Ps, wd	–
Cladonia fimbriata (L.) Fr.	Qr	–
Coenogonium pineti (Schrad.) Lucking & Lumbsch	Qr, Ps, Tc	–
Diarthonis spadicea (Leight.) Frisch, Ertz, Coppins & P.F. Cannon	Qr	–
Evernia prunastri (L.) Ach.	Qr	NT
Fellhanera gyrophorica Sérus., Coppins, Diederich & Scheid.	Qr	LC, Ind.
Fuscidea pusilla Tønsberg	Cb	–
Graphis scripta (L.) Ach.	Cb	NT
Hypocenomyce scalaris (Ach.) M. Choisy	Ps	–
Hypogymnia physodes (L.) Nyl.	Qr	–
Hypogymnia tubulosa (Schaer.) Hav.	Qr	PP, NT
Lecanora argentata (Ach.) Malme	Cb	–
Lecanora carpinea (L.) Vain.	Cb	–
Lecanora expallens Ach.	Qr	–
Lecanora pulicaris (Pers.) Ach.	Cb	–
Lecanora stanislai Guzow-Krzem., Łubek, Malícek & Kukwa	Qr	–
Lecanora thysanophora R.C. Harris	Cb	–
Lepra albescens (Huds.) Hafellner	Cb	–

cd. Tabeli 1

1	2	3
Lepra amara (Ach.) Hafellner	Cb, Qr	–
Lepraria elobata Tønsberg	Cb	–
Lepraria finkii (B. de Lesd. ex Hue) R.C. Harris	Qr, Tc	–
Lepraria incana (L.) Ach.	Bp, Cb, Pt, Ps, Qr, Tc	–
Lepraria jackii Tønsberg	Ps	–
Melanelixia glabratula (Lamy) Sandler & Arup	Qr, Tc	–
Melanohalea exasperatula (Nyl.) O. Blanco et al.	Qr	–
Micarea soralifera Guzow-Krzem., Czarnota, Łubek & Kukwa	Qr, wd	–
Micarea tomentosa Czarnota & Coppins	wd	–
#*Microcalicium disseminatum* (Ach.) Vain.	Qr	Ind.
Parmelia sulcata Taylor	Qr	–
Pertusaria coccodes (Ach.) Nyl.	Cb	NT
Phaeophyscia endophoenicea (Harm.) Moberg	Cb	EN
Phaeophyscia orbicularis (Neck.) Moberg	Cb	–
Phlyctis argena (Ach.) Flot.	Cb, Qr	–
Physcia adscendens (Fr.) H. Olivier	Qr	–
Physcia stellaris (L.) Nyl.	Qr	–
Physcia tenella (Scop.) DC.	Qr	–
Placynthiella dasea (Stirt.) Tønsberg	Qr	–
Platismatia glauca (L.) W.L. Culb. & C.F. Culb.	Qr, Cb	–
Polycauliona polycarpa (Hoffm.) Frödén, Arup & Søchting	Qr	–
Porina aenea (Wallr.) Zahlbr.	Cb	–
Pseudevernia furfuracea (L.) Zopf	Qr	–
Rinodina degeliana Coppins	Qr	–
Rinodina efflorescens Malme	Cb	–
Ropalospora viridis (Tønsberg) Tønsberg	Cb	–
Scoliciosporum sarothamni (Vain.) Vězda	Qr	–
Toniniopsis separabilis (Nyl.) Gerasimova & A. Beck	Cb	EN
Trapeliopsis flexuosa (Fr.) Coppins & P. James	Pt, wd	–
Trapeliopsis granulosa (Hoffm.) Lumbsch	Qr, wd	–
Xanthoria parietina (L.) Th. Fr.	Pt, Qr	–
Xylospora caradocensis (Nyl.) Bendiksby & Timdal	Ps	–

Explanations: # – non-lichenized saprobic fungus; Bp – *Betula pendula*; Cb – *Carpinus betulus*; Ps – *Pinus sylvestris*; Pt – *Populus tremula*; Qr – *Quercus* sp.; Tc – *Tilia cordata*; wd – wood; SP – Strict protection; PP – Partial protection; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; LC – Least Concern; NT – Near Threatened; Ind. – Indicator of lowland old-growth forest.

level of species richness is relatively high. The surrounding forests are highly fragmented, predominantly privately owned and dominated by Scots pine across most habitats.

It should be emphasised that prior to the Second World War, forest cover in this area was considerably lower, and the forests of the present reserve were largely surrounded by arable land (MAPA 1937). At that time, they were privately owned by the Krasiński family entail estate based in Opinogóra, which operated between 1839 and 1944. During the Second World War, the local forests were heavily degraded as a result of exploitative forest management. After the war, following the transfer of larger forest complexes to the State Treasury (State Forests), restoration measures were undertaken and large-scale afforestation of former agricultural land was initiated. These afforestation efforts were carried out mainly using Scots pine, often without sufficient consideration of local habitat conditions. In this context, the presence of several nationally rare and threatened lichen species within the reserve is especially remarkable. Two legally protected species were recorded: *Chrysothrix candelaris* (strict protection) and *Hypogymnia tubulosa* (partial protection). Twelve species are included in the national Red List of lichens, representing the following threat categories:

– CR – 1 (*Chrysothrix candelaris*),
– EN – 2 (*Phaeophyscia endophoenicea, Toniniopsis separabilis*),
– VU – 2 (*Biatora efflorescens*, *Chaenotheca xyloxena*),
– LC – 1 (*Fellhanera gyrophorica*),
– NT – 6 (*Chaenotheca furfuracea*, *Ch. trichialis*, *Evernia prunastri*, *Graphis scripta*, *Hypogymnia tubulosa*, *Pertusaria coccodes*).

Chrysothrix candelaris, *Fellhanera gyrophorica*, and *Microcalicium disseminatum* are stenoecious species regarded as indicators of lowland old-growth forests in Poland (CZYŻEWSKA et al. 2003). In addition, several species that are relatively frequent and not threatened at the national scale but are typically absent from managed forests (FAŁTYNOWICZ et al. 2024) were recorded in the reserve, including *Agonimia repleta*, *Lecanora stanislai*, *L. thysanophora*, *Micarea tomentosa*, *Rinodina degeliana*, and *R. efflorescens*.

The highest lichen species richness within the reserve was recorded on oak trees. The positive role of oaks in shaping epiphytic lichen diversity in forest ecosystems is well documented (CIEŚLIŃSKI and TOBOLEWSKI 1988, KUBIAK and SUCHARZEWSKA 2018, KUBIAK 2020). The exceptional richness of oak-associated lichens results, among other factors, from the long lifespan of individual trees and their occurrence across a wide range of forest community types (DANIELEWICZ and PAWLACZYK 2006). This specificity is also reflected in the fact that oaks typically host the highest number of lichen species exclusive to this phorophyte among all studied tree species (CIEŚLIŃSKI and TOBOLEWSKI 1988, KUBIAK 2013). Hornbeam

also played an important role, reaching several decades of age and forming a second tree layer within the stand. This species was most frequently found together with crustose, moisture-demanding and shade-tolerant lichens, such as *Arthonia radiata*, *Biatora efflorescens*, *Graphis scripta*, *Lecanora thysanophora*, and *Porina aenea*.

A proper assessment of species diversity within a given area requires consideration of a broader spatial context as well as knowledge of the history of anthropogenic landscape transformations. At the national scale, the North Mazovian Lowland is among the regions with a relatively high degree of environmental transformation, with particularly intensive changes occurring over the last 100 years (PLIT 1996). According to the classification proposed by FALIŃSKI (1975), this area corresponds predominantly to degrees V–VI of vegetation synanthropisation on a seven-degree scale. Consequently, the synanthropisation of local vegetation is markedly higher than in the Masurian Lakeland to the north or the North Podlasie Lowland to the east (FALIŃSKI 2015) – regions regarded as one of the centres of lichen biodiversity in Poland (CIEŚLIŃSKI 2003). As a result, the expected species richness of analogous natural objects and vegetation types differs between these regions of Poland. In terms of total species number, the lichen biota of the Paryż Nature Reserve is clearly poorer than that of comparable sites in north-eastern Poland (KOLANKO 2009, KUBIAK 2011a, KUBIAK et al. 2017). However, it includes several particularly noteworthy species that are extremely rare in lowland areas outside this part of the country. These include *Fellhanera gyrophorica* and *Phaeophyscia endophoenicea*, which exhibit relatively distinct and spatially limited centres of distribution in Poland.

Fellhanera gyrophorica is primarily distributed in Central and Eastern Europe (GBIF Secretariat. 2026). In Poland, most known localities are concentrated in the northern and north-eastern regions, where the species inhabits extensive, well-preserved forest complexes. In the eastern part of its range, it occurs mainly on oaks and less frequently on hornbeams and limes within oak-hornbeam forests (KUBIAK 2011b), whereas in western regions it is most often recorded on beech bark in beech forests (KUKWA et al. 2025). Outside this core area, the species has been recorded from only a few localities in central Poland and, more recently, from two sites in the Gorce Mountains, where it was found on beech bark in beech forest communities (CZARNOTA and TANONA 2020). In Poland and Lithuania, *F. gyrophorica* is regarded as an indicator of old-growth primeval forests (MOTIEJŪNAITĖ et al. 2004).

Phaeophyscia endophoenicea is a typical forest lichen preferring natural forest communities from lowland to montane zones. It grows on various deciduous tree species and is occasionally found in old parks. The species typically colonises moss-covered bark or bark enriched with nutrients. In Europe, it is widely distributed but not common (MALÍČEK et al. 2026).

In Poland, the species was until recently known exclusively from southern uplands and lower montane areas (FAŁTYNOWICZ 2003); however, it has since been recorded in light oak-hornbeam forests and beech forests in northern Poland (KUBIAK 2010, 2024, SZYMCZYK 2020, SZYMCZYK et al. 2025).

Considering the distribution of these species in Poland (Figures 2–3), the particular role of the North Mazovian Lowland – and the Paryż Nature Reserve in particular – as a potential connecting area shaping their current distribution ranges becomes apparent. In this context, the North Mazovian Lowland represents a particularly important and promising research area. The region may substantially contribute to improving knowledge of the actual distribution ranges of certain lichen species, as well as to a better understanding of their sensitivity to varying degrees of anthropogenic environmental transformation across different parts of the country. The reserve is located in the immediate vicinity of the ecological corridor

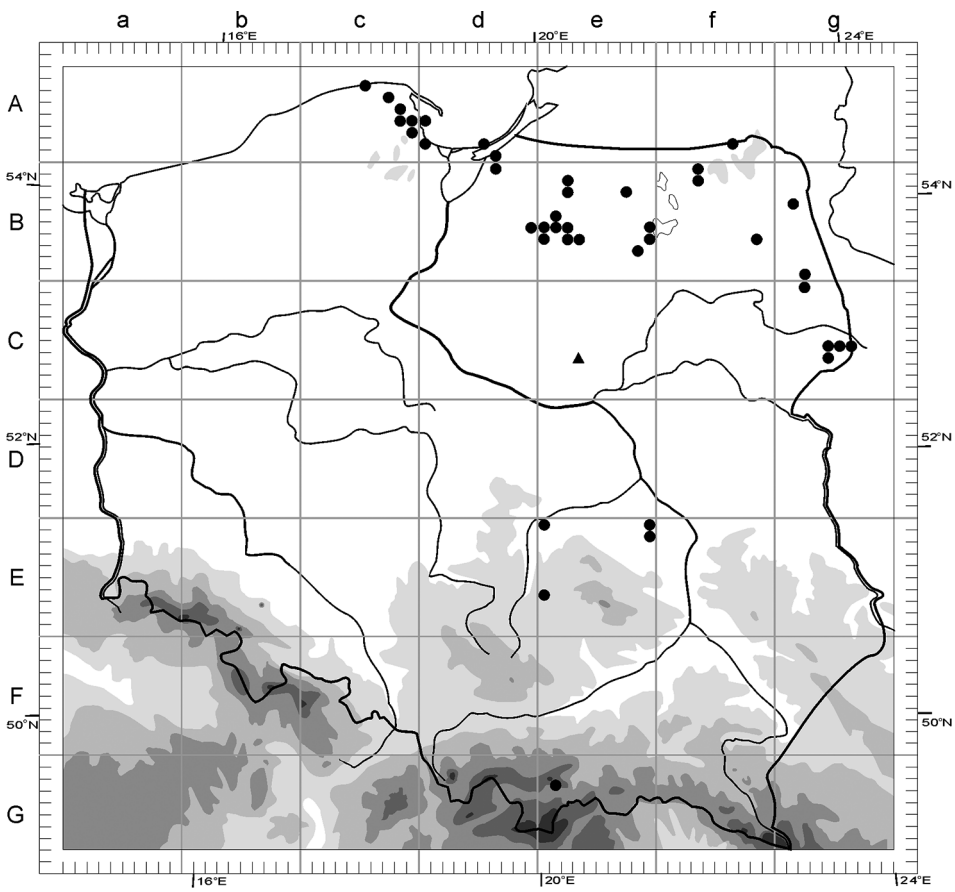


Fig. 2. Distribution of *Fellhanera gyrophorica* sites in Poland according to the ATPOL grid (● – known sites, ▲ – new site in the Paryż Nature Reserve)

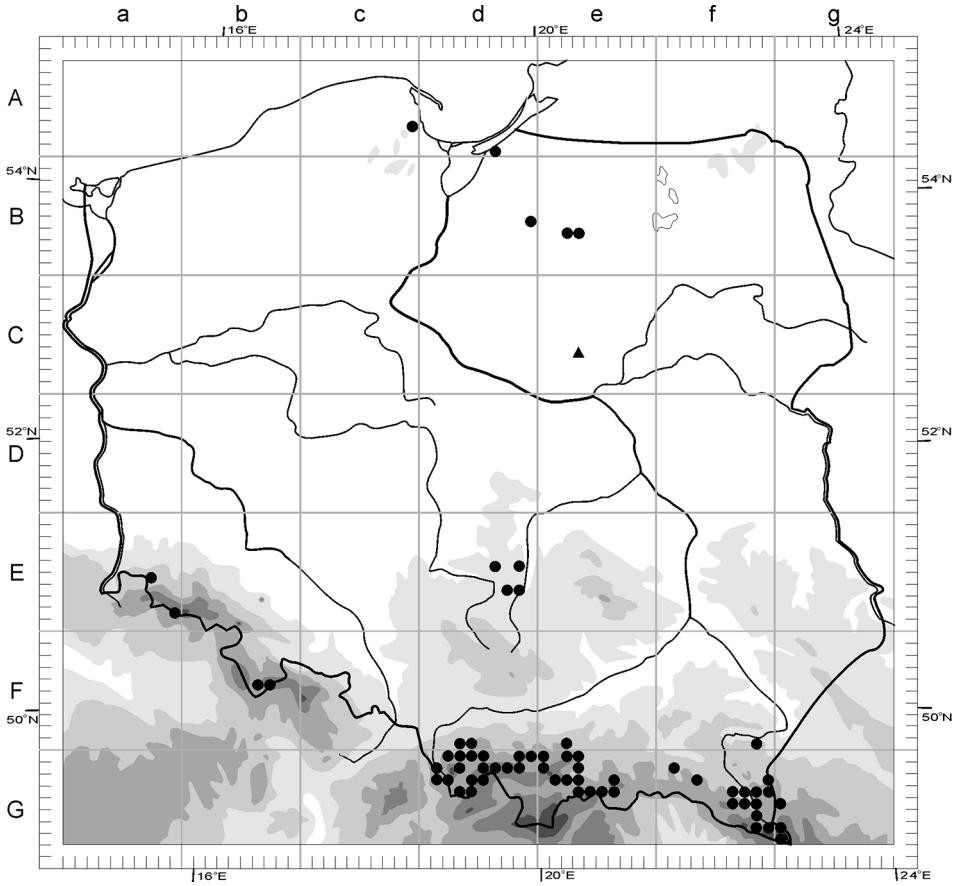


Fig. 3. Distribution of *Phaeophyscia endophoenicea* sites in Poland according to the ATPOL grid (● – known sites, ▲ – new site in the Paryż Nature Reserve)

of the Wkra River Valley (ECOLOGICAL CORRIDORS 2012). This corridor is of national importance and connects valuable natural areas within the Górzno-Lidzbark Landscape Park in the northwest with areas associated with the Vistula River valley in the southeast (STRZELECKI 2015). Unfortunately, despite the extension of corridor boundaries in 2012 compared to the 2005 version, the Paryż Nature Reserve was not included within its limits, which appears unjustified in light of the lichenological results presented here. The corridor should also encompass the second newly established reserve in the Płońsk Forest District, Pieńki Rzewińskie Nature Reserve, through which the corridor currently runs but covers only a small part of its area.

Conclusions

The results obtained, including the presence of several nationally rare and threatened lichen species, demonstrate the above-average natural value of the studied area and confirm the validity of establishing a nature reserve at this site. Nevertheless, further research on lichens is recommended. Such studies should cover areas of the reserve that have not yet been surveyed, as well as its buffer zone, and should also incorporate quantitative assessments of species occurrence. It is important to evaluate the current state of the local lichen assemblage in the context of its dynamic status. The assemblage may represent a remnant of a formerly more diverse assemblage that is currently declining. Alternatively, it may be in a phase of recovery following the cessation of certain negative factors, such as the establishment of an in situ conservation area and the reduction of air pollution. Finally, it is possible that the lichen assemblage is in a period of relative stagnation, in which the positive effects of improved air quality do not lead to an increase in species diversity due to specific habitat limitations. In this context, analysing the functional traits of lichens, including morphological, physiological, and reproductive traits such as growth form, photobiont type, and secondary metabolites (ELLIS et al. 2021), may prove especially useful. These traits influence species responses to environmental change and can help interpret patterns in species occurrence and sensitivity to anthropogenic pressures.

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ECOLOGICAL MARINE ECOTOURISM SUITABILITY ANALYSIS OF KELONG PANCING MADU TIGA IN SENGGARANG BESAR WATERS, BINTAN REGENCY, INDONESIA

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K e y w o r d s: canoeing, fishing, floating accommodation, floating seafood culinary, shuttle boating.

A b s t r a c t

This study evaluates the ecological suitability of the Kelong Pancing Madu Tiga (KPMT) area in Senggarang Besar Waters, Bintan Regency, Indonesia, for the development of marine ecotourism. Field surveys conducted between August 2024 and January 2025 assessed environmental and ecological parameters across three stations, including wave height,

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sediment type, current speed, seawater depth and clarity, temperature, pH, freshwater availability, the presence of dangerous biota, and fish abundance. Suitability was quantified using the Ecotourism Suitability Index (ESI), supported by statistical comparison of physical-chemical variables. Results showed that ecological conditions in the KPMT area ranged from suitable to very suitable for a variety of marine ecotourism activities. Activities such as offshore stays, fishing with fishermen, bedroom-front fishing, shuttle boating, canoeing, and seafood-based tourism consistently achieved ESI values between 83.33 % and 100 %, indicating strong ecological potential. Key drivers of suitability included calm wave conditions, high fish abundance, safe biotic environments, and favorable water quality parameters. Although some spatial variation was observed – particularly in current speed, clarity, and temperature – these remained within acceptable thresholds for sustainable ecotourism. Overall, the findings confirm that KPMT possesses high ecological feasibility for continued ecotourism development, while also highlighting the need for careful environmental management and strategic promotion to ensure long-term sustainability.

Introduction

Ecotourism is a form of sustainable tourism that emphasizes responsible travel to natural areas, aiming to educate travelers while promoting environmental conservation and the well-being of local communities. Marine ecotourism involves activities related to marine activities carried out under or above the sea surface. This approach not only seeks to minimize the negative impacts of tourism but also strives to balance ecological preservation with economic benefits for local populations (STARCZEWSKI et al. 2018, SEERVI 2023, BAYDENIZ et al. 2024). Ecotourism focuses on protecting natural ecosystems and biodiversity, involves local communities in tourism planning and benefits, ensuring their needs are met, aims to maintain and respect local traditions, provides employment opportunities for residents, and contributes to long-term economic growth without compromising environmental integrity (RAKHMONOV 2024, HOJCSKA et al. 2025).

Ecotourism is a form of responsible travel to natural areas that aims to conserve environmental resources, enhance the well-being and empowerment of local communities, and promote environmental education. It is underpinned by principles that seek to minimize negative ecological impacts, generate direct socio-economic benefits for host communities, foster respect for local communities, and contribute to the long-term conservation of natural heritage. A destination may be formally classified as ecotourism only when it fulfills five core criteria: it is nature-based, ecologically sustainable, incorporates environmental education, provides tangible benefits to local communities, and ensures a high-quality visitor experience consistent with conservation objectives (RAMÍREZ and SANTANA 2019, DE GROSBOIS and FENNNEL 2021).

Ecotourism suitability index (ESI) analysis involves evaluating various environmental and ecological parameters to determine the potential of coastal and marine areas for tourism activities. This analysis is crucial

for sustainable tourism development, ensuring that tourism activities do not damage the environment while providing economic benefits. A suitability analysis using a tourism suitability matrix was used by SAHRI et al. (2022) in Tanjung Kelapa. This method involves mathematical calculations and field data to assess suitability for beach recreation, snorkeling, and diving. The analysis results showed varying suitability at various stations, with some areas considered unsuitable for diving. The tourism suitability index (TSI), more specifically for ecotourism areas using the terminology of the ecotourism suitability index (ESI), was also applied at Tamiang Beach Bay, Pulau Laut Tanjung Selayar, Kotabaru, South Kalimantan. This study considered ten parameters, including seawater depth and clarity, to evaluate suitability. The analysis revealed high suitability scores at all stations, indicating a favorable environment for marine tourism. Tamiang Bay area was found to be very suitable for snorkeling and beach activities, with a significant tourist capacity, supporting sustainable tourism development (HUSEIN et al. 2024).

Kelong refers to a set of traditional fishing gear consisting of a wooden house built above sea level and equipped with a fish-trapping net under the house. The building is erected on poles embedded in the seabed. Kelong houses can function as permanent residences or as residential areas for fishermen (LIM 2017). Kelongs, originally used only as traditional fishing gear, is now utilized by the community as a marine tourism destination. The use of kelongs for marine ecotourism is in line with the shift in tourist interest from old tourism to new tourism, namely managing and seeking specific, natural, and biodiverse ecotourism destinations. Types of natural ecotourism in the kelong area include: fishing and harvesting fish catches with fishermen in the kelong, enjoying the natural beauty at dusk and morning, fishing, overnight stays at sea, swimming, canoeing, and others.

Kelong Pancing Madu Tiga (KPMT) is a tourist destination in Tanjungpinang City, built in 2021 and beginning operations in December 2023. This 3.1-hectare area features a restaurant offering a variety of seafood dishes, a fishing area, water sports, accommodation, and various other facilities. The location of KPMT is very strategic and easily accessible to residents and tourists. It is located approximately 600 meters from the shoreline of Senggarang Besar. To reach Kelong Pancing, visitors can use boats provided by KPMT management. Ecotourism suitability analysis is crucial for the development of sustainable tourism. It is essential to ensure that tourism activities are economically viable, while not damaging the environment and providing economic benefits to the surrounding community. This analysis is essential for the KPMT. This research aimed to assess the environmental suitability of the Senggarang Besar waters for marine ecotourism activities based on water quality parameters and ecological indicators.

Materials and Methods

Time and place

This research was conducted from August 2024 to January 2025 in the Kelong Pancing Madu Tiga (KPMT) area, Senggarang Besar Waters, Tanjungpinang City, Riau Islands Province, Indonesia (Figure 1). Sampling was conducted in the second week of August (sampling 1) 2024, the second week of September 2024 (sampling 2), and the second week of January 2025 (sampling 3). Data were collected from three stations. The KPMT layout is L-shaped. Station *A* is located at the base of the L, at the eastern end of the landing pier. Station *B* is located 150 m to the western end of the KPMT, parallel to the landing pier. Station *C* is located at the apex of the L, 150 m from the arrival pier. These station locations are chosen to represent the station's water conditions and the needs of marine ecotourism activities.

Suitability parameter measurements

Suitability parameters measured included wave height, sediment profile, current speeds, seawater depth, seawater clarity, distance to freshwater stock, presence of dangerous biota, fish population abundance, seawater temperature, and seawater pH. Wave height was measured by measuring the height of the waves above the normal sea surface. Sediment profiles were determined by taking seabed sediments and visually identifying their type, whether sand, sand mixed with coral, muddy sand, or all mud. Current speeds were measured using a current meter. Seawater depth was measured by extending a Secchi disk to the seabed. Seawater clarity was measured using a Secchi disk, and the results were converted to present values. Distance to freshwater stock was measured by pulling a meter line, and freshwater stock was determined by checking the size of the freshwater tank. The presence of dangerous biota was determined by observing water conditions and interviewing fishermen around the location, tourist workers, or ordinary people (YULIANDA et al. 2019, INS 2008, RICE and BRIDGEWATER 2012, MER, 2014).

Fish abundance was calculated using the underwater visual census (UVC) method, referring to SAMOIYS and CARLOS (2000). Using an underwater camera, the entire coral reef was photographed. These images were arranged using UVC software to cover the entire reef area at that location. Then, using the same software, the number of species and fish individuals was counted. Coral community coverage, the number of life form types at the coral, the coral reef depth position, and the flat expanse of the coral reef were

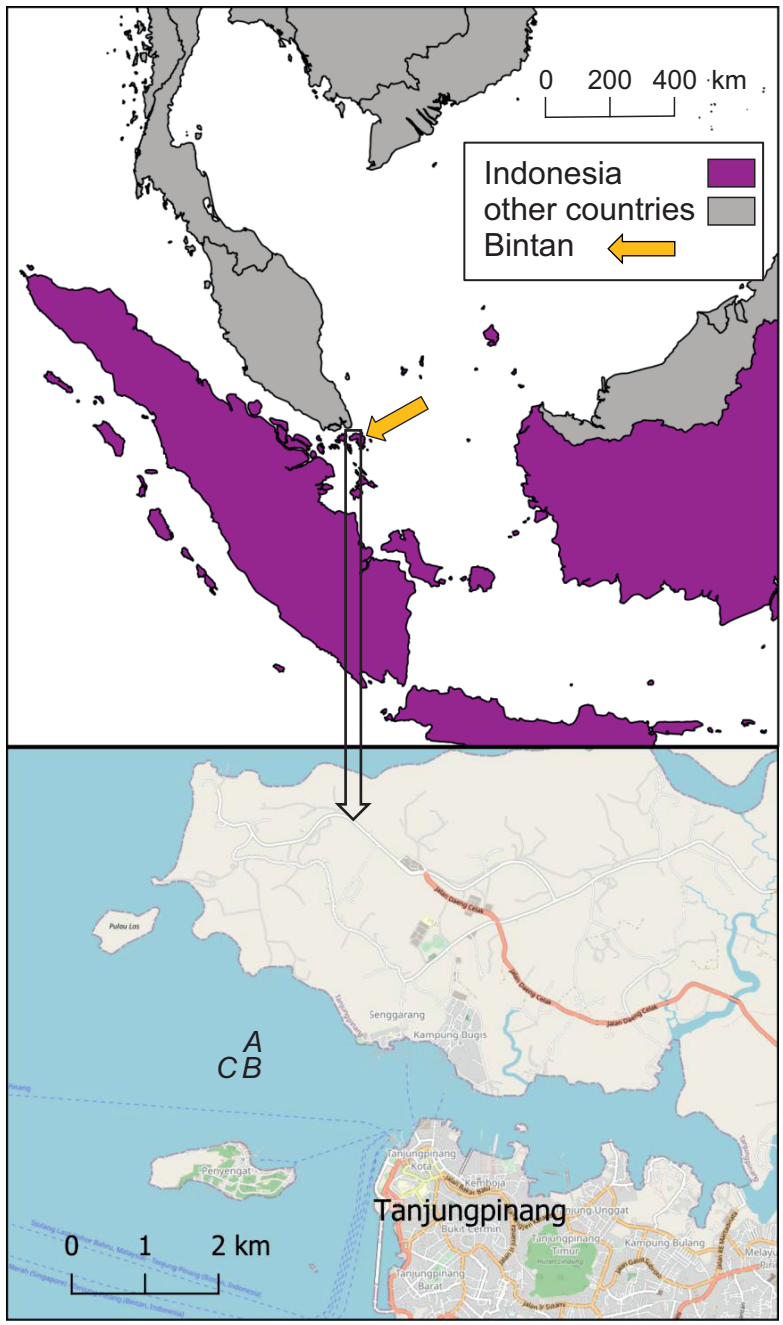


Fig. 1. Study area, Senggarang Besar Waters, Bintan Regency, Indonesia. *A*, *B*, and *C* represent the location of sampling

measured using the underwater photo transect (UPT) method, referring to SHAO et al. (2024). The photographs taken using an underwater camera were then analyzed using the Coral Point Count with Excel extension (CPCe) program, referring to KOHLER and GILL (2006). Meanwhile, seawater temperature and seawater pH were measured using a water checker kit (Reed Instruments R3525 pH Meter, 0.00 to 14.00 pH, -1999 to 2000 Mv, Auto Temp Compensation, 32 to 122 Deg F).

Ecological analysis of ecotourism suitability index

Analysis of the ecotourism suitability index (ESI) used a suitability matrix compiled based on the importance of each parameter to support the ecotourism activities of the area. Each parameter is given a weight and value with reference to YULIANDA et al. (2019), WITHANAGE et al (2024), and TAMBUNAN et al. (2013). Based on field conditions, the ecotourism suitability analysis parameters around the KPMT are wave height, sediment profile, current speeds, seawater depth, seawater clarity, distance to freshwater stock, presence of dangerous biota, fish abundance, seawater temperature, and seawater pH (Table 1). The ecotourism suitability index was determined using a formula adapted from the Marine Tourism Suitability Index assessment method:

$$ESI = \sum [N_i/N_{max}] \cdot 100\ \%$$

where:

ESI – ecotourism suitability index [%]
N_i – value of the i-th parameter (value x score)
N_{max} – the maximum value of a type/category of ecotourism activity

Note: very suitable (100 %); suitable (75–100 %); conditionally suitable (50–75 %); not suitable (<50 %). Value (V) is a suitability weighting constant that refers to YULIANDA et al. (2019), WITHANAGE et al (2024), and TAMBUNAN et al. (2013).

Table 1

Ecotourism suitability index (ESI) parameters, ranges, scores, and categories around KPMT

Parameters	Range	Value (V)	Category
1	2	3	4
Wave height [cm]	< 25	3	very suitable
	25–50	2	suitable
	50–75	1	condit. suitable
	> 75	0	not suitable

cont. Table 1

1	2	3	4
Sediment profile	sand	3	very suitable
	sandy coral	2	suitable
	muddy sand	1	condit. suitable
	mud	0	not suitable
Current speeds [m/sec.]	0–0.15	3	very suitable
	0.15– 0.30	2	suitable
	0.30–0.50	1	condit. suitable
	> 0.50	0	not suitable
Seawater depth [m]	< 5	3	very suitable
	5–15	2	suitable
	15–20	1	condit. suitable
	> 25	0	not suitable
Seawater clarity [%]	> 80	3	very suitable
	50–80	2	suitable
	20–50	1	condit. suitable
	< 20 0	0	not suitable
Distance to freshwater stock [km]	< 0.5	3	very suitable
	> 0.5–1	2	Suitable
	> 1–2	1	condit. suitable
	> 2	0	not suitable
Freshwater stock [ton]	> 15	3	very suitable
	10–15	2	suitable
	5–10	1	condit. suitable
	< 5	0	not suitable
Presence of dangerous biota	None	3	very suitable
	pufferfish	2	suitable
	sting ray	1	condit. suitable
	shark, crocodile	0	not suitable
Fish abundance [per 100 m^2]	very much (> 100)	3	very suitable
	many (50–100)	2	suitable
	little (20–50)	1	condit. suitable
	very little (< 20)	0	not suitable
Seawater temperature [^oC]	> 30	3	very suitable
	20–30	2	suitable
	10–20	1	condit. suitable
	< 10	0	not suitable
Seawater pH	8	3	very suitable
	7	2	suitable
	6	1	condit. suitable
	5	0	not suitable

Source: own elaboration based on: YULIANDA et al. (2019), WITHANAGE et al (2024), and TAMBUNAN et al. (2013)

Data analysis

The data obtained were tabulated, analyzed using Welch's independent samples *t*-test (*p*-values), ESI, and discussed descriptively with reference to theories and previous research results related to the results of this study. Due to the presence of both quantitative and qualitative variables, statistical analysis was conducted exclusively on measurable physical-chemical parameters, while descriptive analysis was applied to categorical attributes. Pairwise comparisons among stations were performed using Welch's independent samples *t*-test to account for small sample sizes and potential heterogeneity of variances, with a significance level set at $p < 0.05$.

The suitability of ecotourism for staying in the middle of the ocean was analyzed by measuring and weighting some parameters: wave height, current speeds, seawater clarity, seawater temperature, seawater depth, presence of dangerous biota, distance to freshwater stocks, and quantity of freshwater stocks. The KPMT is also equipped with a fishing net under its hull. The fishing gear is operated by professional personnel from the KPMT. This activity can involve a limited number of visitors, including preparing the gear, lowering it into the water, waiting, lifting it, harvesting, cleaning the catch, cooking, and consuming it. The selection of these parameters is based on technical considerations of suitability (attractiveness, economics, safety, and conservation) for fishing with the fishermen. The suitability of ecotourism for this activity was analyzed by measuring wave height, current speeds, seawater depth, and fish population abundance. Fishing in front of the bedroom was analysed by measuring and weighting some parameters: wave height, sediment profile, current speeds, seawater depth, presence of dangerous biota, fish population abundance, fish biodiversity index, distance to freshwater stocks, and quantity of freshwater stocks.

Water tours around the waters of Senggaran Besar are quite popular with tourists. They use a shuttle boat with a capacity of 50 people, lasting 1–2 hours per trip. Four parameters have been selected based on technical considerations of the suitability of shuttle boating around Senggarang Besar waters. The parameters analysed were wave height, current speeds, seawater depth, and the presence of dangerous biota. For canoeing around KPMT, measurements and weighting some parameters, such as wave height, sediment profile, current speeds, seawater depth, seawater clarity, seawater temperature, seawater pH, presence of dangerous biota, distance to freshwater stocks, and quantity of freshwater stocks. An ecological analysis of the suitability of seafood culinary tourism was carried out by measuring wave height, current speeds, and the presence of dangerous biota.

Results

General conditions of the research location

Senggarang Besar is a coastal area on Bintan Island, part of Senggarang Village, Bintan Regency, Riau Archipelago Province, Indonesia. Most residents of Senggarang Besar work as fishermen, with settlements located along the coast and along the harbor. KPMT is a marine ecotourism area that covers 3.31 hectares in these waters. This ecotourism resort employs more than 25 workers, both permanent and contract, ensuring smooth operations. Its organizational structure consists of a resort manager, operational leaders and members, housekeeping leaders and members, chefs, cooks, cook helpers, stewards, receptionists, baristas, waiters, security, engineering, boat captains and crew, administrators, and maintenance operators.

All tourism activities (Figure 2) consistently emphasize the importance of human love for nature. This tourist area is smoke-free, clean, and healthy living is encouraged, and tourists are strictly prohibited from throwing trash into the sea. They are also constantly reminded to conserve fresh water and minimize electricity use. This tourism business activity also involves the surrounding community, for example, by involving them as permanent workers, and utilizing agricultural commodities, fisheries, livestock, and other products from the local community. This tourism business also respects the culture and religion of the surrounding community. In other words, KPMT's marine ecotourism business contributes to the development of the local economy.

Accommodation facilities

KPMT offers three types of bedrooms, each equipped with different amenities: Sea Standard Room, Sea Deluxe Room, and Sea Villa Room. The type, number, size, and amenities of each room type are listed in Table 2.

Table 2

Number, size, and amenities of each room type at KPMT, Senggarang Besar, Bintan Regency, Indonesia

Room type	Number (units)	Area [m^2]	Facilities
Sea standard room	10	17	bed, air conditioning, bathroom, television, and wi-fi
Sea deluxe room	10	20	bed, air conditioning, bathroom, television, and wi-fi
Sea villa room	10	41	bed, air conditioning, bathroom, television, and wi-fi

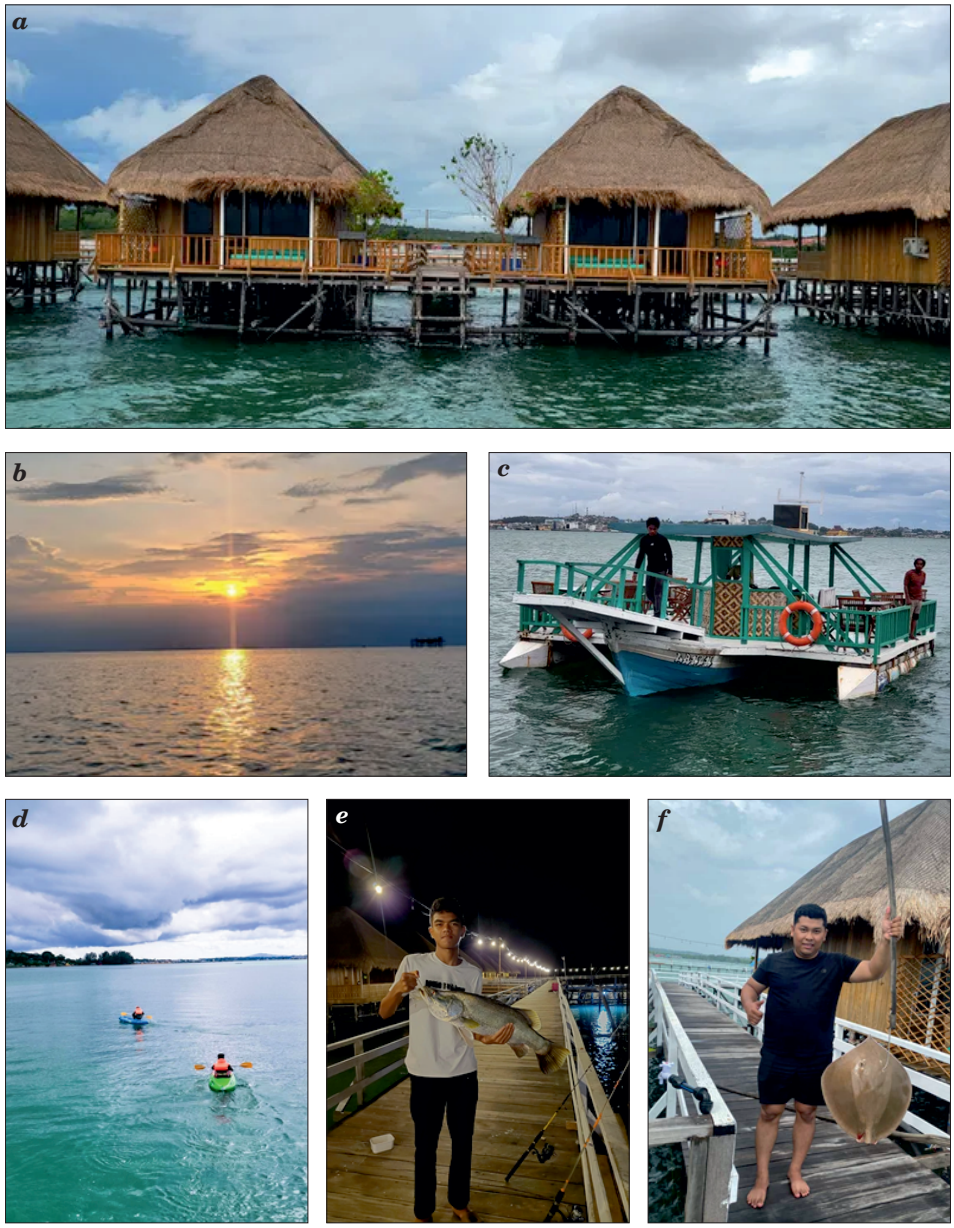


Fig. 2. Some ecotourism activities in KPMT, Senggarang Besar, Bintan Regency, Indonesia:
a – sea villa room view; *b* – sunset view; *c* – shuttle boat;
d – canoeing activity; *e, f* – fishing activities

Marine ecotourism activities in KPMT

KPMT is a popular marine tourism destination and a tourist icon for Tanjung Pinang City. Inaugurated as a tourist attraction on December 23, 2023, KPMT features fishing facilities with an abundance of various fish populations, offering stunning natural beauty with expansive ocean views, clear, and calm waters. The area also features comfortable accommodations, a restaurant serving fresh seafood, and various other amenities. Some of these activities are: experience staying in the middle of the ocean, experience watching the sunset, experience fishing with the fishermen, cook and eat the catch, fishing in front of the bedroom, or any position in KPMT, shuttle boating around Senggarang Besar waters, canoeing around the KPMT, and seafood culinary tourism.

Suitability analysis of ecotourism

Detailed results for each parameter are presented in Table 3. Quantitative ecotourism suitability parameters were statistically compared among the three research stations (*A*, *B*, and *C*) in the KPMT area of Senggarang Besar waters, Bintan Regency.

Significant differences in wave height were observed between Stations *A* and *C* ($p < 0.05$), with Station C exhibiting higher values, whereas no significant differences were detected between Stations *A* and *B* or *B* and *C*. Current speed varied significantly across all station comparisons ($p < 0.05$), with the highest velocities consistently recorded at Station *C*. In contrast, seawater depth did not differ significantly among stations ($p > 0.05$), indicating relatively uniform bathymetric conditions throughout the study area.

Seawater clarity showed significant spatial variation, with clarity values increasing from Station *A* to Station *C*. All pairwise comparisons revealed statistically significant differences ($p < 0.05$), suggesting heterogeneous light penetration conditions among stations. Seawater temperature differed significantly between Station *A* and Stations *B* and *C* ($p < 0.05$), with Station *A* displaying lower mean temperatures, while no significant difference was observed between Stations *B* and *C*. Similarly, seawater pH values were generally stable across stations; however, a significant difference was detected between Stations *A* and *C* ($p < 0.05$), with Station *C* presenting slightly higher pH values.

Qualitative parameters, including sediment type, presence of dangerous biota, freshwater availability, and fish population abundance, were uniform across all stations. Sediments were dominated by sand, no dangerous biota

Table 3

Ecotourism suitability parameters in KPMT, Senggarang Besar waters, Bintan Regency, Indonesia

Parameters	Research stations		
	A	*B*	*C*
Wave height [cm]	18	20	23
	22	24	24
	24	23	24
Sediment profile	sand	sand	sand
	sand	sand	sand
	sand	sand	sand
Current speeds [m/second]	0.09	0.13	0.28
	0.08	0.12	0.19
	0.06	0.14	0.18
Seawater depth [m]	4.6	4.5	4.5
	4.1	4.5	4.6
	4.4	4.5	4.8
Seawater clarity [%]	60	70	80
	50	70	85
	55	60	82
Distance to freshwater stock [km]	0.5	0.5	0.5
	0.7	0.8	0.9
	0.7	0.8	0.9
Freshwater stock [ton]	10	10	10
	10	10	10
	10	10	10
Presence of dangerous biota	none	none	none
	none	none	none
	none	none	none
Fish abundance	very much	very much	very much
	very much	very much	very much
	very much	very much	very much
Seawater temperature [ºC]	29.7	30.8	31.2
	29.2	31.1	30.1
	29.3	31.4	30.4
Seawater pH	8.1	8.2	8.4
	8.1	8.1	8.3
	8.1	8.1	8.2

were recorded, freshwater availability was adequate, and fish populations were consistently described as very abundant, indicating favorable ecological conditions for marine ecotourism activities.

Overall, the statistical analysis demonstrates that while several key environmental parameters vary significantly among stations – particularly current speed and water clarity – these variations remain within acceptable ecological thresholds for ecotourism development. The combination of statistically favorable physical conditions and consistently positive biological indicators suggests that the KPMT area possesses strong potential for sustainable marine ecotourism.

The Ecotourism Suitability Index (ESI) is associated with activities around the kelong, such as staying in the middle of the ocean, and fishing in front bedroom. The analysis was conducted by considering some ecotourism suitability index parameters. This analysis is necessary to determine whether the KPMT tourist area meets the standards for marine tourism.

Experience staying in the middle of the ocean

The suitability of ecotourism for staying in the middle of the ocean was analyzed by measuring and weighting some parameters. The selection of these parameters is based on technical considerations of suitability of attractiveness, economics, safety and conservation. The values of each of these parameters were then analyzed using a suitability index (Table 4). This analysis is also used to assess the feasibility of the experience of enjoying the melancholy atmosphere at sunset.

The Ecotourism Suitability Index (ESI) for staying in the middle of the ocean in the KPMT area ranged from 85.71 % to 90.48 %, indicating that all research stations were classified as *suitable* for staying in the middle of the ocean. Station *C* recorded the highest ESI value (90.48 %), followed by Station *B* (88.10 %) and Station *A* (85.71 %). High suitability across stations was primarily driven by favorable wave height, current speed, seawater temperature, and the absence of dangerous biota, all of which consistently achieved high scores. Variations in ESI values among stations were mainly attributed to differences in seawater clarity, fish abundance, and seawater depth, with Station *C* showing more optimal ecological conditions. Overall, the results indicate that the KPMT offshore area possesses strong potential for sustainable marine ecotourism development.

Table 4

Analysis of ecotourism suitability index (ESI) for staying in the middle of the ocean in KPMT, Senggarang Besar waters, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Current speeds [m/sec.]	3	3	3	3	9	9	9
Seawater clarity [m]	2	2	3	2	4	4	6
Seawater temperature [°C]	3	3	3	1	3	3	3
Fish abundance around the coral reef [individuals per 100 m^2]	1	2	2	1	1	2	2
Seawater depth [m]	3	3	2	1	3	3	2
Presence of dangerous biota	3	3	3	1	3	3	3
Distance to freshwater stock [km]	2	2	2	1	2	2	2
Freshwater stock [ton]	2	2	2	1	2	2	2
Value of the i-th parameter (N_i) for staying in the middle of the ocean in KPMT					36	37	38
The maximum value (N_{max}) for staying in the middle of the ocean in KPMT					42	42	42
Ecotourism suitability index (N_i / N_{max} · 100 %)					**85.714**	**88.095**	**90.476**

Explanations: very suitable (100 %); suitable (75–100 %); conditionally suitable (50–75 %); not suitable (<50 %).

Experience fishing with the fishermen

The KPMT is also equipped with a fishing net under its hull. The fishing gear is operated by professional personnel from the KPMT. This activity can involve a limited number of visitors, including preparing the gear, lowering it into the water, waiting, lifting it, harvesting, cleaning the catch, cooking, and consuming it. Some parameters have been measured, and an ecological analysis of the suitability of this tourism activity was carried out, as shown in Table 5.

The ESI for fishing activities with local fishermen in the KPMT area showed high suitability across all research stations. Stations A and B achieved the maximum ESI value of 100 %, classifying them as *very suitable*, while Station *C* recorded an ESI of 88.89 % and was categorized as *suitable*. High suitability at Stations *A* and *B* was driven by optimal wave height, current speed, seawater depth, and high fish population abundance. The slightly lower ESI at Station *C* was mainly influenced by reduced current speed, although overall conditions remained favorable for fishing-based ecotourism (Table 5).

Table 5

Analysis of ecotourism suitability index (ESI; %) for fishing with the fishermen in KPMT, Senggarang Besar waters, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Current speeds [m/sec.]	3	3	2	3	9	9	6
Seawater depth [m]	3	3	3	1	3	3	3
Fish population abundance	3	3	3	2	6	6	6
Value of the i-th parameter (N_i) for fishing with the fishermen in KPMT					27	27	24
The maximum value (N_{max}) for fishing with the fishermen in KPMT					27	27	27
Ecotourism suitability index (N_i / N_{max} · 100 %)					**100**	**100**	**88.889**

Fishing in front of the bedroom or from any position in KPMT

The suitability of ecotourism for fishing in front of the bedroom was analysed by measuring and weighting some parameters. The selection of these parameters is based on technical considerations of the suitability of this activity. The values of each of these parameters were then analysed using a suitability index (Table 6).

Table 6

Analysis of ecotourism suitability index (ESI) for fishing in front bedroom or other place in KPMT, Senggarang Besar waters, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total Score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Sediment profile	3	3	3	2	6	6	6
Current speeds [m/sec.]	3	3	2	3	9	9	6
Seawater depth [m]	3	3	3	1	3	3	3
Presence of dangerous biota	3	3	3	1	3	3	3
Fish abundance	3	3	3	2	6	6	6
Value of the i-th parameter (N_i) for fishing in front of the bedroom in KPMT					36	36	33
The maximum value (N_{max}) for fishing in front of the bedroom in KPMT					36	36	36
Ecotourism suitability index (N_i / N_{max} · 100 %)					**100**	**100**	**91.667**

The Ecotourism Suitability Index for fishing in front of the bedroom or nearby areas in the KPMT waters indicated very high suitability across all research stations. Stations *A* and *B* achieved maximum ESI values of 100 % and were classified as *very suitable*, while Station *C* recorded an ESI

of 91.67 % and was categorized *as suitable*. High suitability at Stations *A* and *B* was driven by optimal wave height, favorable sediment conditions, appropriate seawater depth, absence of dangerous biota, and high fish abundance. The slightly lower ESI at Station *C* was mainly due to reduced current speed, although overall conditions remained favorable for nearshore fishing ecotourism (Table 6).

Shuttle boating around Senggarang Besar waters

Water tours around the waters of Senggaran Besar are quite popular with tourists. They use a shuttle boat with a capacity of 50 people, lasting 1–2 hours per trip. Four parameters have been selected based on technical considerations of the suitability of shuttle boating around Senggarang Besar waters. An ecological analysis of the suitability of this ecotourism is presented in Table 7.

Table 7

Analysis of ecotourism suitability index (ESI) for shuttle boating around Senggarang Besar waters, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Current speeds [m/sec.]	3	3	2	3	9	9	6
Seawater depth [m]	3	3	3	1	3	3	3
Presence of dangerous biota	3	3	3	1	3	3	3
Value of the i-th parameter (N_i) for shuttle boating around Senggarang Besar waters					24	24	23
The maximum value (N_{max}) for shuttle boating around Senggarang Besar waters					24	24	24
Ecotourism suitability index (N_i / N_{max} · 100 %)					100	100	95.833

The ESI for shuttle boating around Senggarang Besar waters showed very high suitability across all research stations. Stations *A* and *B* achieved maximum ESI values of 100 % and were classified as *very suitable*, while Station *C* recorded an ESI of 95.83% and was categorized as *Suitable*. High suitability was supported by favorable wave height, adequate seawater depth, and the absence of dangerous biota. The slightly lower ESI at Station *C* was mainly influenced by reduced current speed, although overall conditions remained suitable for shuttle boating operations (Table 7).

Canoeing around the KPMT

The suitability of ecotourism for canoeing around KPMT was analysed by measuring and weighting some parameters. The values of each of these parameters were then analysed using a suitability index (Table 8).

Table 8

Analysis of ecotourism suitability index (ESI) for canoeing around KPMT, Senggarang Besar waters, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Sediment profile	1	1	1	1	1	1	1
Current speeds [m/sec.]	3	3	2	3	9	9	6
Seawater depth [m]	3	3	3	1	3	3	3
Seawater clarity [m]	2	2	3	1	2	2	3
Seawater temperature [°C]	3	3	3	1	3	3	3
Seawater pH	3	3	3	1	3	3	3
Fish population abundance	1	1	1	1	1	1	1
Presence of dangerous biota	3	3	3	2	6	6	6
Distance to freshwater stock [km]	3	3	3	1	3	3	3
Freshwater stock [ton]	2	2	2	1	2	2	2
Value of the i-th parameter (N_i) for canoeing around KPMT					42	42	40
The maximum value (N_{max}) for canoeing around KPMT					48	48	48
Ecotourism suitability index (N_i / N_{max} · 100 %)					**87.500**	**87.500**	**83.333**

Canoeing around the KPMT waters classified all research stations as *suitable*. Stations *A* and *B* recorded identical ESI values of 87.50 %, while Station *C* showed a slightly lower value of 83.33 %. High suitability was supported by favorable wave height, appropriate seawater depth, suitable temperature and pH, and the absence of dangerous biota. The lower ESI at Station *C* was mainly influenced by reduced current speed and lower seawater clarity, although overall conditions remained acceptable for canoeing activities (Table 8).

Seafood culinary tourism

KPMT has a restaurant serving a variety of fresh seafood. This restaurant has an area of 150 m^2 and can accommodate up to 100 guests. An ecological analysis of the suitability based on technical considerations of this ecotourism is presented in Table 9.

Table 9
Analysis of ecotourism suitability index (ESI) for seafood culinary tourism in KPMT, Bintan Regency, Indonesia

Parameter	Score (N) per station			Value (V)	Total score ($N \cdot V$) per station		
	A	*B*	*C*		*A*	*B*	*C*
Wave height [cm]	3	3	3	3	9	9	9
Current speeds [m/sec.]	3	3	2	3	9	9	6
Presence of dangerous biota	3	3	3	1	3	3	3
Value of the i-th parameter (N_i) for seafood culinary tourism in KPMT					21	21	18
The maximum value (N_{max}) for seafood culinary tourism in KPMT					21	21	21
Ecotourism suitability index (N_i / N_{max} · 100%)					**100**	**100**	**87.500**

The ESI for seafood culinary tourism in KPMT indicated very high suitability across all stations. Stations *A* and *B* achieved maximum ESI values of 100 % (*very suitable*), while Station *C* recorded 87.50 % (suitable). High suitability was driven by optimal wave height, favorable current speeds, and the absence of dangerous biota. The slightly lower ESI at Station *C* was mainly due to reduced current speed, although overall conditions remained favorable for seafood culinary tourism.

Discussion

This study revealed that ecological suitability index (ESI) levels ranged from suitable (83.333 %) to very suitable (100 %). This is comparable with PUTRA et al. (2018), who assessed the tourism suitability of recreational beaches in Padang City. Their results indicated that 18 beach locations were classified as 'very suitable', with an average suitability index of 82.28 %, while eight locations were categorized as 'moderately suitable', with an average score of approximately 70 %. Only one site, located in front of Bung Hatta University, fell into the 'conditionally suitable' category, with a suitability value of 27 %. For mangrove ecotourism, six locations were identified as 'very suitable', with an average suitability index of 92 %, and nine locations were classified as 'moderately suitable', averaging 73 %. In addition, three locations were considered 'conditionally suitable', with an average score of 49%. In contrast, the Carolina (Pasa Laban) Beach area was categorized as 'not suitable' for mangrove ecotourism, with a suitability value of 33 %.

Similar findings were reported by ANNIDA and BAIHAQI (2025), who studied the marine tourism suitability index and ecological carrying capacity on the west and east coasts of Pangandaran, Indonesia. TSI for marine tourism activities was generally of high suitability on both the West

and East Coasts, with clear spatial differences. Beach recreation exhibits consistently very high suitability, with uniform values of 96.7 % across all stations on the West Coast and slightly lower but still very suitable values of 91.0 % on the East Coast, reflecting optimal coastal conditions for this activity. Swimming suitability on the West Coast ranges from 81.2 % to 90.7 %, classifying the area as quite appropriate to very suitable, whereas the East Coast shows greater variability and lower values (76.4–85.9 %). Camping activities demonstrate the most pronounced contrast between coasts, with the West Coast showing uniformly very suitable conditions (96.7 %) at all stations, while the East Coast records lower and consistent values of 66.7 %. LELLOLTERY et al. (2018) revealed a high marine resource potential in Marsegu Island Nature Tourism Park, Maluku, Indonesia. TSI indicated that the area is suitable for ecotourism, with a snorkeling suitability value of 74.79 %, while diving showed a suitability value of 67.32 %. JOHAN et al. (2017) also reported that Enggano Island had three categories for marine ecotourism suitability of diving categories, namely suitable, quite suitable, and not suitable, while the marine ecotourism of snorkeling had only two categories: suitable and quite suitable.

Ecotourism is responsible travel to natural areas that conserves the environment, supports the well-being of local communities, and promotes environmental education and cultural appreciation. Its key pillars include minimizing negative impacts on ecosystems, providing direct financial benefits and empowerment to local communities, fostering understanding and respect for local cultures, and contributing to the conservation of natural and cultural heritage (DE GROSBOIS and FENNELL 2021, FURGAŁA-SELEZNIOW and JANKUN-WOŹNICKA 2021, BALOCH et al. 2023). There are five fundamental core principles that an area must meet to be considered an ecotourism area: nature-based, ecologically sustainable, environmental education, local benefits, and visitor satisfaction (BUCKLEY 2012, RAMÍREZ and SANTANA 2019). Comparable findings were reported by LEOTAUD et al. (2024), who examined tourism diversification strategies in the Caribbean and highlighted the growing role of ecotourism and community-based tourism within marine and coastal environments as mechanisms for promoting sustainable development. The ecotourism nuance is clearly visible in the KPMT area. Educational aspects regarding the importance of environmental conservation for tourists and the community involved in this activity are prominent. Every visitor is always advised on the importance of environmental sustainability, such as not damaging the ecosystem, using everything sparingly, using water sparingly, disposing of waste properly, and so on. The presence of KPMT also has an impact on the economic development of the surrounding community, from their direct involvement as KPMT employees to the utilization of agricultural products and fish caught in this ecotourism area.

An ecotourism suitability index is a tool used to assess whether a location is suitable for ecotourism development by considering its physical, ecological, and environmental factors. In general, it appears that all ecotourism activities in the KPMT area are feasible to be conducted. The Ecotourism Suitability Index (ESI) for each activity has the following range of numbers: staying in the middle of the ocean for (85.714–90.476 %), fishing in front of the bedroom or elsewhere within the KPMT (93.333–100 %), and canoeing around the KPMT for (87.500–91.667 %).

Safety is a fundamental component of ecotourism development. Even when supporting aspects such as tourist attractions, facilities, and accessibility are well established, they become insignificant if a destination cannot guarantee visitor safety. Ecotourism activities are typically conducted in open and often remote natural environments, where levels of risk and vulnerability are inherently higher. Under these conditions, safety plays a crucial role in safeguarding visitor well-being and ensuring the long-term sustainability of the destination. The primary safety challenges in ecotourism originate from natural environmental factors, including wave height, ocean current velocity, and interactions with potentially dangerous wildlife. As a result, visitors' perceptions of safety strongly influence their trust in the destination, which subsequently affects satisfaction and revisit intention (NEVES 2013, YU et al. 2023, JAWORSKA 2024). In the context of Kelong Pancing Madu Tiga (KPMT), this study demonstrates that safety is consistently prioritized by site managers. All ecotourism activities are carefully planned and implemented by considering environmental conditions, particularly the presence or absence of dangerous animals, wave dynamics, and current speeds. Around KPMT, these aspects include wave height, current speeds, the presence of dangerous biota, and seawater temperature. Waves that are too high can cause canoes to capsize, boats to toss passengers, scatter furniture, and cause passengers to become intoxicated and suffer injuries or illnesses (ZOLJOODI 2017, TRUNG et al. 2020, KIM et al. 2024). Likewise, current speed, where currents that are too strong (> 0.50 m/second) can endanger boat passengers, canoeists, and anglers (PEREIRA et al. 2014, AUGUSTINE et al. 2022, WULANDARI et al. 2024). The presence of dangerous biota such as pufferfish, sharks, sea crocodiles, jellyfish, stingrays, and other dangerous animals is absolutely intolerable (ARIEFIANDA et al. 2019, KELANA et al. 2022). Fortunately, in the KPMT ecotourism area, so far, these dangerous animals have never been found. Best practice combines prevention, capacity building, community participation, and legal safeguards; some studies recommend structured safety systems, regulated standards, and continuous monitoring (GSTAETTNER et al. 2017, FYALL et al. 2022, HILD and JÓHANNESSON 2023). The provision of more comprehensive facilities, together with enhanced safety and security

support, has the potential to improve the suitability of several marine tourism activities, particularly canoeing, by increasing operational safety and visitor confidence.

The comfort aspect of ecotourism is also important (SYUKUR et al. 2021, ASKAR 2023). In the KPMT area, this aspect includes wave height, current speeds, seawater depth, the absence of dangerous biota, and seawater temperature. The comfortable feeling is created by various natural factors in this area. Wave heights are neither too high nor too calm, creating a feeling like riding a swing. Current speeds are also very friendly. Although slow, the movement of the water mass is visible, as if trying to cleanse and replace the existing water with fresh seawater. Furthermore, the seawater depth is very suitable, so visitors don't have to worry about the depth. The seawater temperature is tropical, close to human body temperature. This provides a refreshing feeling for visitors who swim, snorkel, and dive. The absence of dangerous animals certainly adds to the feeling of comfort (AN and SHIN 2019, ARIANI et al. 2020).

The attractiveness of an ecotourism program constitutes a critical determinant of visitor preference and satisfaction, as tourists may be willing to overlook other destination attributes to obtain high-quality experiential outcomes (CAPARRÓS-MARTÍNEZ et al. 2022, ABIDIN et al. 2023, SULISTIOWATI et al. 2023). In the context of ecotourism activities in the KPMT area, this attractiveness is closely associated with biophysical and environmental quality indicators, including sediment profile, seawater depth and clarity, fish population abundance, coral community cover, diversity of coral life forms, reef depth positioning, reef flat extent, seawater temperature, and pH. Comparable evidence was reported by AGIUS et al. (2019), who analysed Mediterranean island destinations and demonstrated that marine ecotourism – particularly within coastal and marine protected areas – has considerable potential to meet the expectations of specialist ecotourist markets. Their findings further emphasize that the quality of ecotourism settings is a key driver of ecotourist satisfaction. Moreover, in island environments subject to substantial anthropogenic pressure, optimal ecotourism sites tend to be located in peripheral coastal and marine zones, with marine ecotourism emerging as the most appropriate ecotourism product for balancing tourism development and environmental conservation.

The availability of ecotourism facilities and infrastructure is certainly no less important (EFFENDI et al. 2022, TJILEN et al. 2022). In the KPMT ecotourism area, this factor includes the availability of freshwater for tourists, namely the distance to freshwater stocks and the quantity available. During the study, the distance (0.5–0.9 km) and amount of fresh water supplies (10 tons) were considered sufficient for the needs of tourists (YULIANDA et al. 2019). Furthermore, the availability of canoes (ten units), speedboats

(two units), and other facilities is also crucial. The equipment and supplies for this activity were considered more than adequate. This equipment can also be rented from other parties nearby if more is needed. Nevertheless, the long-term development of marine ecotourism requires carefully planned and systematically implemented facilities and infrastructure to ensure environmental sustainability and the economic viability of the ecotourism sector.

These ESI figures are still significantly suitable, suggesting that ecotourism activities can still be developed through appropriate marketing strategies. For example, strengthening environmental awareness through ecotourism. Marketing through collaborations with tourism operators from other countries, social media promotions, and holding environmental awareness events such as mangrove planting, turtle hatchling releases, and so on (MAUGERI et al. 2017, EFFENDI et al. 2025). An important consideration is the influence of seasonal variability. During the northern monsoon period (December–February), the study area is characterized by stronger wind regimes, increased rainfall, elevated wave heights, and reduced water clarity. These conditions may temporarily limit the suitability and safety of certain marine ecotourism activities, indicating the need for seasonal management and adaptive planning.

Conclusion

The KPMT area has been developed through an integrated approach that emphasizes environmental conservation, educational functions, and the empowerment of surrounding local communities. The analysis of the ecotourism suitability index (ESI) at each research station for each ecotourism activity ecologically shows a suitability level ranging from suitable to very suitable. This figure shows that ecotourism activities in the area can still be developed through appropriate planning. Marketing through collaborations with tourism operators from other countries, social media promotions, and holding environmental awareness events is recommended. Further research is necessary to strengthen the sustainable management and development of the ecotourism area. Future studies should focus on identifying the dominant ecotourism attractions that contribute most significantly to visitor interest, as well as examining how individual ecotourism activities are perceived and experienced by tourists. In addition, systematic evaluation of promotional strategies is needed to determine their effectiveness in increasing visitor awareness, satisfaction, and visitation rates.

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