Technical report

Deliverable 5.2

Evaluation of potential of crustacean production in RAS in Pomerania

University of Gdańsk

National Marine Fisheries Research Institute

InnoAquaTech

Cross-border development and transfer of innovative and sustainable aquaculture technologies in the South Baltic area

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Contents

1. Introduction

Food from farms are our everyday life. We have become very frequent consumers of aquaculture products, although the products rarely come from our local market and we know little about them, especially when it comes to crustaceans. Their production takes place mainly in Asia. Yet, shrimp farms, which meet the needs of local markets, arise every year in the United States and in Europe. Still, countries of the South Baltic region, including Poland, do not play a major role in the sector. To change the current situation the team from the Institute of Oceanography, University of Gdańsk and National Marine Fisheries Research Institute has taken a challenge to develop the potential of crustacean aquaculture focusing on combination of new species and technologies under the Interreg South Baltic Programme project InnoAquaTech – Cross-border development and transfer of innovative and sustainable aquaculture technologies in the South Baltic area (STHB.01.02.00-DE-0022/15-00).

1.1. Whiteleg shrimp

Whiteleg shrimp *Litopenaeus vannamei* comes from the Eastern Pacific, where the water temperature is usually above 20°C. An adult female lays 100-250 thousand eggs, from which the larvae hatch. A few larval stages appear In the developmental cycle of the species. The larvae develop in estuaries, lagoons or mangrove areas. The last larval stadium transforms into a juvenile individual that migrates to deeper regions in the ocean (up to 70 m), where it grows to about 20 cm in length.

Excellent aquaculture species:

- wide salinity range tolerance (0.5-45 ppt, optimum 15-25 ppt) and wide temperature range tolerance (optimum 26-30^{o}C, lower temperatures up to 15^{o}C tolerance)
- fast growth rate (1.5-3 g / week, max. weight 120 g)
- fish feed of a relatively low protein content (20-35%) in comparison to other species
- relatively low food conversion rate (app 1.2)
- possible farming in various densities, from less than 40 specimens/m2 (semi-intensive) to 400 specimens/m2 (hyperintensive)

- pathogen free flocks in the US guarantee the production of healthy larvae throughout the year, well tolerating a long-distance transport
- a well-known species
- potential for the multitrophic aquaculture.

Fig. 1 ***Litopenaeus vannamei*** **from RAS-500 Institute of Oceanography, University of Gdańsk experimental culture (Photo credits Piotr Kendzierski)**

1.2. Aquaculture in Pomerania, Poland

Promotion of aquaculture, both conventional methods and more importantly RAS (Recirculating Aquaculture Systems), as well as introducing new species and increasing the share of species other than carp in aquaculture overall are the main goals in the Polish aquaculture sector. These have to be achieved together with providing environmental services. Significant production growth to 51,600 tonnes by 2023; including 6,000 tonnes from recirculating aquaculture systems and increased contribution to sustainable employment and environmental protection are expected.

So far in Poland the farming is represented by land-based freshwater farms, mostly carried out in traditional earth ponds. In 2015, total national aquaculture production reached 38,590 tonnes – an 8 % increase compared to 2013. The biggest category is carp production, which amounted to 19,000 tonnes and made up over 50 % of total aquaculture output in 2014. The output of rainbow trout was 15,000 tonnes. Compared to carp, rainbow trout farming is a nascent industry in the country, having started only in the 20th century. The active development of trout farming started at the end of 1990s, and production has been stagnating over the past few years. Trout production is carried out in intensive fish production facilities. Recirculating aquaculture systems start to appear for the production of trout and salmons but also for new species such as tilapia. A review of technology pool has been done within InnoAquaTech project, which shows a growing interest in new, sustainable production technology (Annex 1 – Technology pool).

Although the average annual fish consumption is below the average per capita fish consumption in the EU, there is a growing awareness among Polish consumers regarding fish and seafood consumption. They are choosing fish products with more care and attention, getting information about products, and benefiting from campaigns promoting consumption and the health benefits of fish and seafood. Rising health awareness is indicated to be the most popular reason for fish consumption. In recent years, Poland has observed a dynamic development of sushi bars and restaurants, including major sushi bar chains. National authorities are undertaking a series of measures to increase fish consumption, particularly among young consumers.

1.2.1. Investments into innovative aquaculture solutions and business opportunities

The adopted Operational Programme (OP) is well aligned with the priorities of the European Maritime and Fisheries Fund (EMFF) and the EU's reformed Common Fisheries Policy (CFP). As a result, funds will go to three main areas: supporting the CFP objectives to ensure that fishing and aquaculture activities are or become more environmentally sustainable; boosting the development of aquaculture; and improving livelihoods in local communities that depend on fishing and aquaculture, by creating jobs and alternative sources of income. The programme will also contribute to improving the competitiveness of the fisheries and aquaculture sectors. Finally, funds will support improvements in the management. What is more, there is an increasing demand for various fish products and the processing industry is looking for new suppliers.

Therefore innovative aquaculture based on recirculating systems as well as introduction of new species, such as shrimp *Litopenaeus vannamei* gives a tremendous potential for investors.

1.3. Demonstrating the sustainability and raising awareness for the possibility of crustacean production in RAS systems in Pomerania, Poland

Aim of the study was to evaluate the potential of crustacean production in RAS in Poland: The aim was achieved through: laboratory study focused on a mini-scale RAS white leg shrimp *Litopenaeus vannamei* cultivation and the experiments on growth and nutritional value of the shrimp, and socio-economic study regarding consumer perception and identification of scientific market knowledge.

1.3.1. Laboratory study - Growth and nutritional value of *Litopenaeus vannamei* from the small-scale laboratory culture

Two RAS min-systems, each consisting of three main units: culture shrimp tanks (500 liters), filters (mechanical and biological filters, mechanical protein skimming with ozone, UV sterilization, submersible pumps) with a control system (electronic devices) and water preparation tank (250 liters), were installed in the laboratory of the Institute of Oceanography, University of Gdansk in the end of June 2017. Both systems have closed water flow, and the only difference is biological filtration: a trickle filter, and fluidized moving filtration media.

During trail 1 (November 2017 – March 2018) around 550 shrimps were placed in each RAS (T = 17 °C, S = 33 PSU). They were gradually (3 weeks) acclimatized to a higher temperature (25 °C) and lower salinity (29 PSU). During the whole experiment they were fed six time per day with the known portion of commercial feed (0.8, 1.2 and 1.5 mm pellets, Scretting, Norway). Water parameters such as temperature, salinity, pH and redox potential were also controlled six times a day, as were the behavior, mortality and number of molts. Ammonia, nitrite, nitrate, phosphates and silicates were determined three times a week. Weight (± 0.001 g) and length (± 1 mm) of randomly collected shrimps (10 from each tank) were determined every second week. The whole experiment was carried out for 16 weeks. After this time, the shrimp were stunned (i.e. rendered unconscious) by chilling in ice water. Some of them were transferred to the laboratory of National Marine Fisheries Research Institute for the studies of sensory attributes (color, smell, taste, texture) and weight loss in flesh during freezing. Remaining shrimps were frozen to other analyzes, i.e. elemental composition (carbon, hydrogen and nitrogen contents) and calorific value which was performed at the University of Gdansk and nutritional value and level of chemical contaminants was performed by a project partner – National Marine Fisheries Research Institute.

During trial 2 (August 2018 - March 2019) around 550 shrimps in each RAS (T = 17 °C, S = 33 PSU) were farmed. They were gradually acclimatized to a higher temperature (25 °C) and lower salinity (29 PSU). During the whole experiment they were fed six time per day with the known portion of commercial feed. Water parameters such as temperature, salinity, pH and redox potential were also controlled six times a day, as were the behaviour, mortality and number of smolts. Ammonia, nitrite, nitrate, phosphates and silicates were determined three times a week. Weight (± 0.001 g) and length (± 1 mm) of randomly collected shrimps were determined every second week. The following analysis were performed: determination of phosphate, nitrate, nitrite, silica and ammonia concentrations in water samples from the RAS-500 system for the 2nd trial of white leg shrimp breeding experiment, measuring the rate of excretion of ammonia in shrimp from the RAS-500 system for the white leg shrimp breeding experiment, morphological analysis of the cultivated shrimps/ analysis of the health status of farmed shrimps from the 2nd breeding cycle, analysis of the cultivated shrimps in terms of physico-chemical parameters, growth rate and nutritional value of *Litopenaeus vannamei*, analysis of elemental composition CHN in abdomen muscle of shrimps available on the Polish market and experimental RAS breeding, analysis of energy value and share of organic matter in the abdomen muscle of shrimps available on the Polish market and experimental RAS breeding.

2. Growth and nutritional value of *Litopenaeus vannamei* in the small-scale laboratory culture

2.1. Materials and methods

2.1.1.Description of experimental set-up RAS-500

2.1.1.1. Purpose of the RAS-500 system

In June 2017 a small scale RAS laboratory facility for shrimps cultivation was installed at the laboratory of the Institute of Oceanography, University of Gdansk for the purpose of the

InnoAquaTech project, pilot study 2 – Farming shrimp in Poland: Increasing the potential of recirculating aquaculture systems.

The RAS-500 has been specifically designed and installed by Aqua Medic Poland Bartosz Blum for the purpose of cultivating shrimps in closed recirculating system (The unit is able to treat ca. 500-1000 l. and the shrimp tank volume is ca. 500 l, hence the name RAS-500) and to conduct experiments which will determine how different factors in various combinations effect survival, basic physiological processes, protein content and weight gain of the pacific white shrimp. Simultaneously data for the recommendation of the facility set up and shrimps cultivation were collected and processed. For over four months the facility was tested and calibrated before the juvenile shrimps came for the cultivation and tests in November 2017 (Fig. 2).

Fig. 2 RAS-500 – laboratory at the Institute of Oceanography, University of Gdansk (Photo credits: Basia Dmochowska)

Fig. 3 Transport box of *Litopenaeus vannamei* at the Institue of Oceanography laboratory UG, and a shrimp specimen (Photo credits: Monika Normant-Saremba)

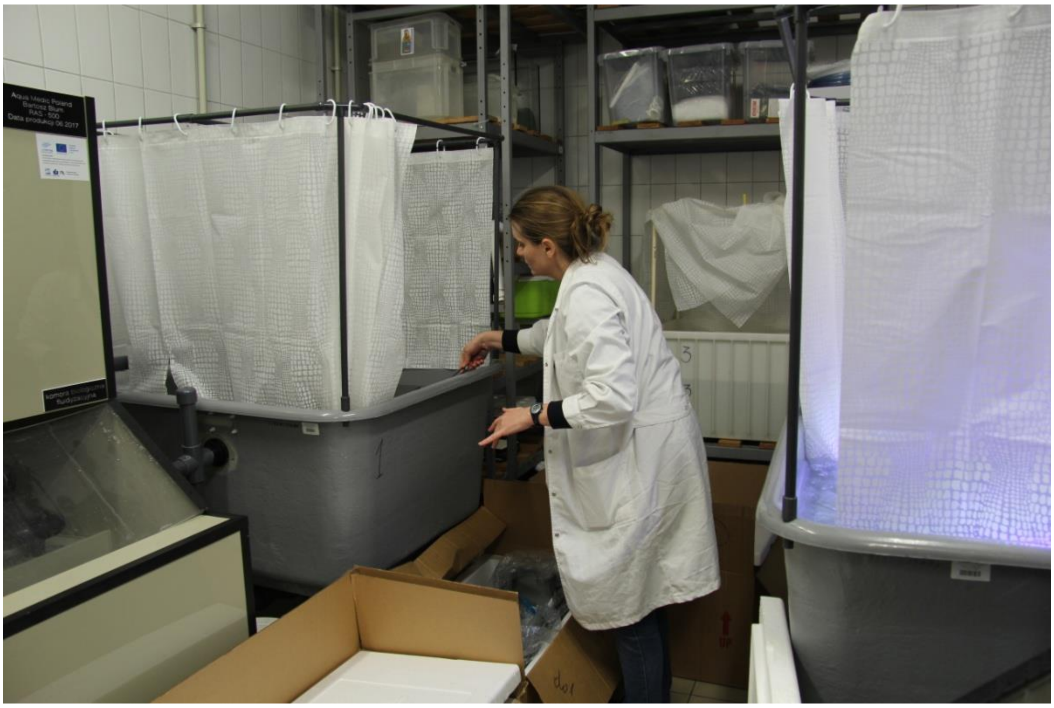


Fig. 4 InnoAquaTech team member working on shrimps transport into the RAS system (1) (Photo credits: Monika Normant-Saremba)

2.1.1.2. System design

RAS-500 (Fig. 5) consists of 3 separate tanks: water preparation tank, main unit (containing: electric cabinet, electronic cabinet, mechanical filtration, protein skimmer, UV and ozone sterilization, biological chamber, heating, filter sump, aeration) and a shrimp tank.

Fig. 5 RAS-500 system design (Photo credits: Basia Dmochowska)

There are two sets of the equipment specified and the sets differ in the biological filtration systems. Biological filtration in RAS 1 is typical wet/dry filtration (trickle filter). Biological filtration in RAS 2 is based on fluidized media fully submerged in the water column. Both systems are used simultaneously. The two sets work independently. It allows us check both filtration systems.

The middle chamber in the RAS sump is designed to install, if needed, various chemical media. In the first RAS, equipped with trickle filter, the outlet from the biological chamber is located in the bottom of the chamber, so that filtration media is not submerged. In the second RAS, equipped with fluidized moving filtration media, the outlet from the biological chamber is located is the top, so that filtration media remains all the time submerged.

The RAS has been designed in such a way, that evaporation of water is limited to minimum. The whole system has been designed and manufactured from corrosion and sea-water resistant materials. Gravity is used for water flow. Filter sump is located at the bottom of the RAS and the main two return pumps pump the water to the top of the unit. The rest of the flow has been designed is such a way, that no other pumps are needed. This helps to save on electricity cost for pumping of the water. Treated water flows into the shrimp tank. From the tank water flows back to the RAS sump through surface and bottom outflows. From the shrimp tank, water flows to the main unit. After mechanical cleaning of the water, the water flows to the skimmer chamber. After skimming, the water flows through the middle sump chamber. The last chamber is the main pump chamber. The pumps pump the water back to the biological chamber (Fig. 6).

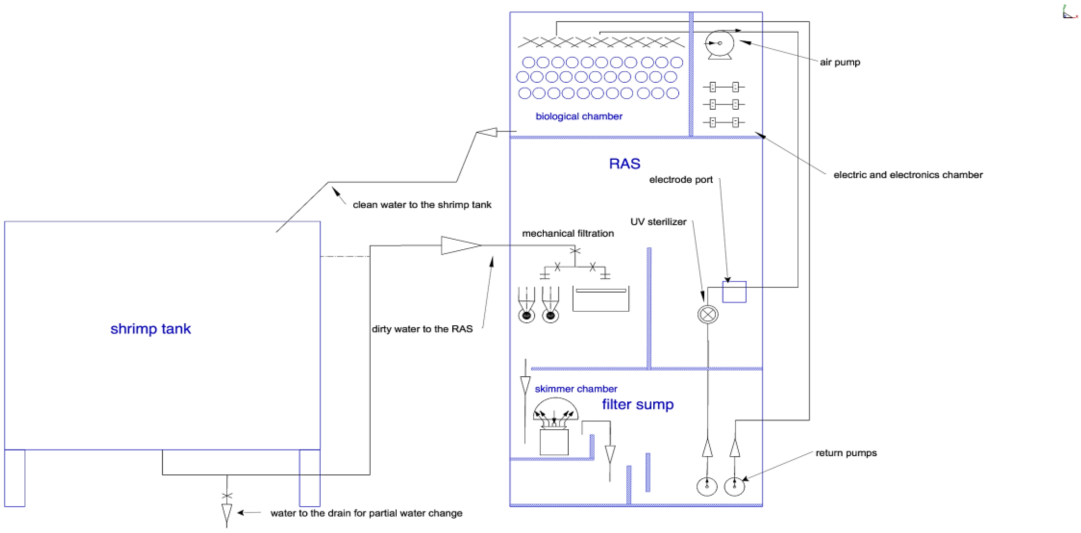


Fig. 6 RAS-500 water flow (Diagram: Aqua Medic Poland Bartosz Blum)

For continuous control and monitoring of basic water parameters the system uses Aqua Medic AT-Control. The system is able to monitor and control through its programmable sockets temperature, pH, redox, and density.

2.1.2. Experimental conditions

Light

To control light condition laboratory was blacked out and the window was curtained. The light: dark regime 14h:10h was controlled with the use of an automatic digital timer. The light source in the laboratory was a lamp with a fluorescent tube.

To increase the Vit. D content in the shrimp soft tissue, in the second trial, digital timer controlled the light regime of aquatic lamp Qube 50 High Power LED, directly above the tank.

Water parameters were constantly monitored.

Temperature

Laboratory temperature was set to 18°C in the first trial and 20°C in the second trial. Laboratory temperature was controlled by the EBERLE air conditioner, located under the ceiling. The optimum temperature in water tanks was gained by the use of an aquatic titanium heater in the main unit of RAS 25±0.2°C. The temperature was set to 20°C and increased to 25°C every few weeks. Water temperature in the water preparation tank (additional reservoir) was controlled by Weipro automatic aquarium heater.
Easy Home Quigg dehumidifier was used to control the air moisture level in the laboratory.

Salinity

Water salinity was maintained at 28. The Tropic Marin® ZooMix Sea Salt, with relatively high cations content: Ca^{2+} and Mg^{2+} was used (Tab. 1, Atkinson and Bindgman 1997).

Tab. 1 Commercial sea salts content. SW- sea water; TM - Tropic Marin; MW – mol weight (g/mol) (Atkinson and Bindgman 1997)

	SW	TM	MW
Salinity	35	32,64	
Major Cations (mmol kg-1)			
Na^{+}	470	442	23.0
K^{+}	102	9.1	39.1
Mg^{2+}	53	46	24.3
Ca^{2+}	20,3	9.1	40.1
Sr^{+}	0,09	0.08	87.6
Sum	607	561	
Major anions (mmol kg^{-1})			
Cl^{-}	550	521	35.5
SO_4^{2-}	28	21	32.1
TCO2	1.90	1.10	12.0
TB	0.42	0.36	10.8
Sum	608	541	

Water in the supplementary compartment was prepared to refill RAS water when necessary. The salinity of additional water was gained by the addition of sea salt to distilled water.

RedOx

To balance redox conditions aquatic air pumps were used in biological filtration compartment of RAS main unit and in cultivation tanks. Also, water in the supplementary compartment was aerated. For protein removal from water, a skimmer was used. The skimmer chamber was cleaned once or twice a day. Ozone supply increases the efficiency of the protein skimmer significantly and raises ORP potential (redox) in water. Ozone minimizes the level of pathogens but also supports biological filtration. Ozone generator with ozone production of 100 mg/h was used.

To improve water redox additional filtration medium – synthetic filter floss was placed in the 100 microns felt fine filter bag.

pH

Distilled water was used for RAS water preparation for proper pH of the environment. To decrease pH - AquaCID Zoolek or 1M HCl solutions were added.

Nutrients maintenance and measurements

In the period prior to shrimps arrival (app. 3 months) one bacteria strains dose (ampoule) PRODIBIO BIODIGEST and ½ nourishment dose (ampoule) PRODIBIO – BIOPTIM were added to the cultivation tanks every two weeks. Later on a blend of bacterial strains, BACTO Start COLOMBO was poured directly on the biofilter in dose 100 ml per tank every week. During cultivation, a dose of 1 ml for every 5 litres of tank water was applied.

Rowa Phos PO_4^{3-} and SiO_2 dsorbent deposit was placed on a fine sieve so that its water flow was directed through the deposit before entering the biofilter compartment.

Nutrient (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, SiO_2) concentration was controlled every two days in the first trial, and every week in the second trial. The following methods were used: Ammonia with Salicylate Method 10031, Nitrate with Cadmium Reduction Method 8039, Nitrite with USEPA Diazotization Method 8507, Phosphate with USEPA1,2 PhosVer 3® (Ascorbic Acid) Method 8048, Silicate with Silicomolybdate Method 8185 (Powder Pillow Tests, HACH LANGE); UV-VIS spectrophotometer DR 600, Hach Lange, Poland.

Acclimation

PLs were sent in the water of the following conditions: salinity ca. 33-36, the temperature ca. 18 °C, pH 7.6, hyperoxia, and ammonia, nitrite, nitrate levels close to zero. PLs were transferred to the laboratory and left in transporting bags and containers for the stabilization of temperature and salinity conditions, for ca. 8h. Every 30min 0.5l of water was added to each bag. The farming temperature in the first trial was obtained by increasing water temperature of in the system by 1 °C every 3 days. In the second trial, the farming conditions were obtained after the final transfer of PLs to RAS systems. The temperature was increased by 1 °C every week.

Fig. 7 Photographs of delivered boxes with shrimps (Photo credits: Basia Dmochowska)

Feeding

Shrimps were hand-fed 6 times a day (7:00; 10:00; 13:00; 16:00; 19:00; 22:00) with dry feed. For PL prawns the feed was grained. Grained feed was soaked in the dose of tank water before being provided to shrimps. In the second trial live food – brine shrimp larvae (Artemia salina) was given as the first feed. More mature shrimps were fed with a granulated feed from an automatic feeder Sera precision Feed A Plus.

In the first trial Gemma Diamond 0.8; 1.2; 1.5mm feed (Scretting, Norway)(Tab. 2), a non GMO product according to EC regulation 1829/2003, was used. In the second trial, CreveTec PL 1000 (Creve Tec Grower 1A, Eric De Muylder, Belgium) was used. The daily amount of feed was dosed according to the Scretting feed protocol.

Brine shrimp for PLs were cultured on site in the water from RAS (from eggs – one teaspoon of dry eggs for 1.5l of saltwater).

Tab. 2 Parameters and composition of feeds used in trials

	Size (mm)	Proteins (%)	Lipids (%)	Ash (%)	Fiber (%)	Phosphorus (%)	Moisture (%)	HUFA (%)	Cholesterol (%)
GEMMA Diamond	0.8-1.5	57	15	10.5	0.2	1.6	No inf.	No inf.	No inf.
CreveTec PL1000	1.0-1.5	min 54	min 12	max 10	max 1	min 1,2	max 10	min 1,6	min 0,5

2.1.3. Cultured animals

Shrimp larvae for both experimental cultures were imported by an aquarist company Aqu Medic Poland Bartosz Blum (Poland) from the Shrimp Improvement Systems (Florida, USA) hatchery, which is a world's leading company producing genetically improved and specific pathogen free *L. vannamei* shrimp broodstock. Larvae for the first trial were obtained on 16.11.2017, whereas for the second one on 08.08.2018. In each case their number was 1000. Size of shrimps from first and second delivery differed considerably (Fig. 8 A, B). Moreover, there was also high inter-individual variability in size among shrimps from the same delivery (Fig. 8 C).

A

B

C

Fig. 8. Initial look of the larvae of *L. vanammei* used in the first (A, C) and second (B) trial.

After 18 (trial 1) and 32 (trial 2) weeks of the culture all shrimp were killed by chilling in an ice slurry. As many as 25 (trial 1) and 25 (trial 2) of them were used for analyses of organoleptic properties and then the rest was preserved differently for further analyses. In the case of trial 1, 22 shrimps were preserved in 4% formaldehyde for health status analyses and 119 were frozen (- 18 ^{o}C) for analyses of energy value, organic matter content and contents of carbon, hydrogen and nitrogen and flesh quality. In the case of trial 2, 28 shrimps were preserved in Davidson fixative (ethanol, formaldehyde, acetic acid, glycerol and water) for health status analyses and 200 were frozen (- 18 ^{o}C) for analyses of energy value, organic matter content and contents of carbon, hydrogen and nitrogen and flesh quality.

2.1.3.1. Shrimp health status

In the first stage, preserved shrimps from trial 1 and 2 were analyzed macroscopically to find out whether there were any lesions present in cephalothorax, abdomen, antenna and antennules, rostrum, eyes, carapace, abdomen, pereiopods and pleopods, telson and gills (Johnson, 1995) indicating fungal, viral or bacterial infections. Next, it was checked microscopically if there were any abnormalities in the respiratory (analysis of the gills), digestive (analysis of the stomach, intestine and hepatopancreas) and circulatory (hemocel, heart) systems. For this purpose, a histological technique described by Larsson et al. (2018) was used.

2.1.4. Analyses of shrimps' growth and nutritional value

2.1.4.1. Length and weight measurements

Length and fresh mass of shrimps cultured in trial 1 and 2 shrimps were determined every second or third week over 15 and 29 weeks respectively. Due to the much smaller size of the individuals imported to the trial 2, the analysis of their length and mass was started in the eighth week (to avoid mortality caused by injury). Length and mass measurements were performed at the same time of the day using 20 randomly taken individuals, 10 from each RAS (Fig. 9). The shrimps were delicately removed from the water onto a paper towel, their length was measured (± 1 mm) from the rostrum to the end of telson (Fig. 10) and then their fresh mass was determined (± 0.001 g). After the measurements, they returned to the breeding tanks. Results were used to determine length-fresh mass relationship according to the power function $y = ax^b$, where y is fresh mass and x is length.

Fig. 9. Shrimps (n=10) taken randomly for measurements from one RAS.

Fig. 10 Measured length (red line) of *L. vanammei*

2.1.4.2. Energy value and organic matter content

After thawing, the length and fresh mass of each shrimp were determined analogously as described in subsection 2.2.4.1. Then the abdominal muscle of each shrimp was dissected (Fig. 11), weighted (± 0.001 g) and lyophilized for 24 h.

Fig. 11 Dissected abdominal muscle of *L. vanammei*

After this time the dry mass was determined (± 0.001 g) and abdominal muscles were separately homogenized in the mortar (Fig. 12).

A

B

Fig. 12 Lyophilized abdominal muscle of *L. vanammei* before (A) and after homogenization in a mortar (B)

The energy value was determined using a modified Phillipson KMB-2 type microbomb calorimeter, which was at first calibrated using benzoic acid samples with a known caloric content (Prus, 1970; Grodzinski et al., 1975). A small portion of the homogenized sample (20-40 mg) was formed into a pellet which was then weighted (± 0.001 g) and placed on platinum mat. Next, the prepared sample was placed in the microbomb which was pressurized with excess pure oxygen (20 atm). Temperature rise caused by combustion heat was recorded. For each shrimp sample three replicates were performed. Energy value (Q) expressed as joules per milligram of dry weight (J mg^{-1} DW) or per milligram of ash-free dry weight (J mg^{-1} AFDW) was calculated based on the formula:

$Q = (K \cdot \Delta T)/m$

where:
K – calibration factor (K = 201.66 and 200.81 J $^{o}C^{-1}$ for trial 1 and trial 2 respectively),
m – pellet weight (mg),
ΔT – temperature difference before and after combustion (^{o}C).

The organic matter content in abdominal muscle of *L. vannamei* was determined after ashing at 450 ◦C for 12 h as the mass loss before and after burning (Gnaiger and Bitterlich, 1984).

2.1.4.3. Carbon, hydrogen and nitrogen contents

Carbon (C), hydrogen (H) and nitrogen (N) contents were determined with a Perkin-Elmer PE CHNS/O 2400 Series Elemental Analyzer (PERKIN ELMER, USA) following the procedure described in Wood (1997) and Burska (2010). Small (2-3 mg) portion of homogenized sample (prepared before for energy value determination) was combusted at a pressure of about 1 atm at a temperature of about 1600-1800 ºC. Proportion of carbon, hydrogen and nitrogen in analyzed sample was calculated based on the products of gaseous oxidation (CO_2, H_2O and N_2) determined by means of a catharometer after passing their mixture to the chromatography column (Bobrański, 1979). Prior to the analyses the device was calibrated using a standard (Acetanilide, C = 71.09%, H = 6.71%, N = 10.36%). For each shrimp sample three replicates were performed. Contents of carbon, hydrogen and nitrogen were expressed in percentage of dry weight (% DW).

2.1.4.4. Organoleptic properties

The length (± 1mm) and fresh mass (± 0.001 g) of each shrimp used for analyses were measured and then they were stored in plastic packaging without ice at a temperature of 1-3°C. The changes in organoleptic properties of shrimps were analyzed in 1st, 3rd, 5th and 7th day of storage for both, raw and cooked (for 5 minutes in 55°C) shrimps using the scoresheets prepared by Torry Research Station (Archer, 2010). The parameters analyzed in raw shrimp were: flesh colour and opacity, head colour, body colour, connective tissue and odour. If the analysed batch was already headed than the head colour parameter was omitted. In cooked shrimps, the following parameters were analysed: cooked odour, flavours and texture. The features of above-mentioned parameters were rated from 5 to 1 for raw shrimp (Table 3) and from 5 to 0 for cooked shrimp (Table 4). For most of the batches three replicates were performed and the average score has been presented in the figures. In the case of smaller batch only 2 or 1 replicates were performed. The half of the analyzed batch from trial 1 was frozen, storage in -20°C for 5 months and, than defrosted and analyzed in the same way as unfrozen batch.

Table 3. Scoresheet for raw shrimps (Archer, 2010)

Score	Flesh colour and opacity	Head	Brown shrimp body	Connective tissue	Odours
5	Translucent	Light brown/ greythrough transparent casing	Pale grey, darker grey spots	Grey	Fresh seaweed; delicate; watery
4	Slight opacityto- wards anterior	Slight green- ing or yel- lowing	Grey/slight mauve tinge. Pale yellow ventrally	Duller grey	Seaweed; sweet; milky; fresh cut grass; metallic; io- dine
3	Opacity extended half way along flesh from anterior end which is beginning to turn yellow	Blackening			Tangy/ sherbet lemon; "sharp"; musty-mouldy leaves; hay
2	May still show translucency at tips of tail. Anterior: yel- low/green	Grey/green/ yellow			Compost; silage; rotting vegetables; humus; muddy; ditch-water; NH_3
1	All opaque	Covered in large green/black blotches. Blackening of head ex- tending along the body		Yellowing	Urinal; doggy; amines; byres; sweaty; NH_3

Table 4. Scoresheet for cooked shrimps (Archer, 2010)

Score	Cooked Odours	Flavours	Texture
5	Chestnuts; seaweed; tangy	"Characteristic"; milky/sweet	Firm, chevy
4	Milky-sweet - condensed milk; fresh urine; mealy; caramel; malty; mushroom sheds/dank	"Characteristic"; milky/sweet - creamy; meaty	
3	Leaf mould; peat; boiled vege- tables	Still slightly sweet; sour/sweet greasy/fatty	
2	Chlorine; sweaty; pow- der/plaster/cement; soapy water - clothes washing; ma- nure; amines; NH_3	Fishy; slightly sour; bland (pink shrimp); may still show very slight sweetness	Lumpy - readily falls into segments which are quite firm
1	Strong sweaty; rotting vegeta- bles; NH_3	Sour; bland; fishy	May still be lumpy but the segments are softer that for 2
0	Strong ammoniacal; faecal; urinal; acrid/scorched cloth; soapy	Soapy; bitter	Very soft; pulpy

2.2. Results and conclusions

2.2.1. Experimental conditions

Shrimp farming water conditions

In 2017 shrimps arrived in twelve boxes (24 bags) in water with the fallowing conditions: salinity 33, temperature 17-18°C. pH during transport dropped to ca. 7.1, despite the addition of ca. 100ml of activated carbon pellets in each bag. In 2019 PLs arrived in one box (2 bags) with the fallowing conditions: salinity 31, temperature 20 °C, pH 7.3.

In the first trial, shrimps were moved to tanks during the first day, in the second trial most PLs were kept in bags for several weeks. The salinity increased during experiments, due to evaporation. The refill with additional water and after an exchange of some water during tanks drainage cleaning de-creased water salinity (Tab. 5). In both trials, the lowest pH was recorded when mineralization of accumulated faeces and feed debris in the drainage occurred. Also after visits (during student classes or workshops), lowered pH was noticed. In both trials, redox in RAS systems exceeded 200mV for most of the time and several times increased above 400mV for a period of time. With an effective skimmer, redox may remain very high, up to 500 mV.

Tab. 5 Farming conditions after acclimation (mean±standard deviation)

	Temperature (˚C)	Salinity	Redox (mV)	pH
RAS A	24.8±0.2	28.9±0.8	271.8±69.0	7.7±0.2
RAS B	24.8±0.2	29.0±0.7	228.1±60.7	7.8±0.1
Bag A-RAS A	23.9±1.3	29.7±1.1	282.3±65.8	7.8±0.2
Bag B	21.2±0.4	29.6±0.5	272.2 ±8.1	7.9±0.2
RAS B	23.8±0.1	29.5±1.7	285.1±58.9	7.8±0.2

Nutrients

The highest risk to cope with during both trials was ammonia poisoning possibility. No high concentration of $NH4^+$ was observed, except for the typical increase noted after the transfer of PLs to tanks (Fig. 13). Due to the almost constant presence of nitrite and nitrate in farming water (Tab. 6), levels of all nitrogen compounds were controlled – and an exchange a significant amount of water in the system was applied, if needed. Such a situation happened in every trial – after cleaning of accumulated feces and feed debris in the drainage (Fig. 14).

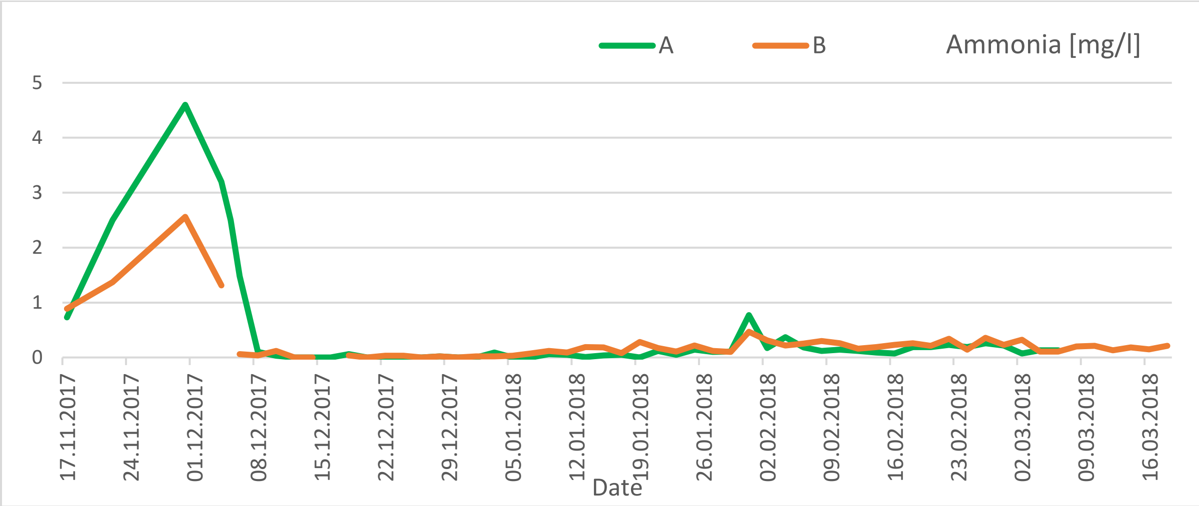


Fig. 13 Ammonia in the 1st trial

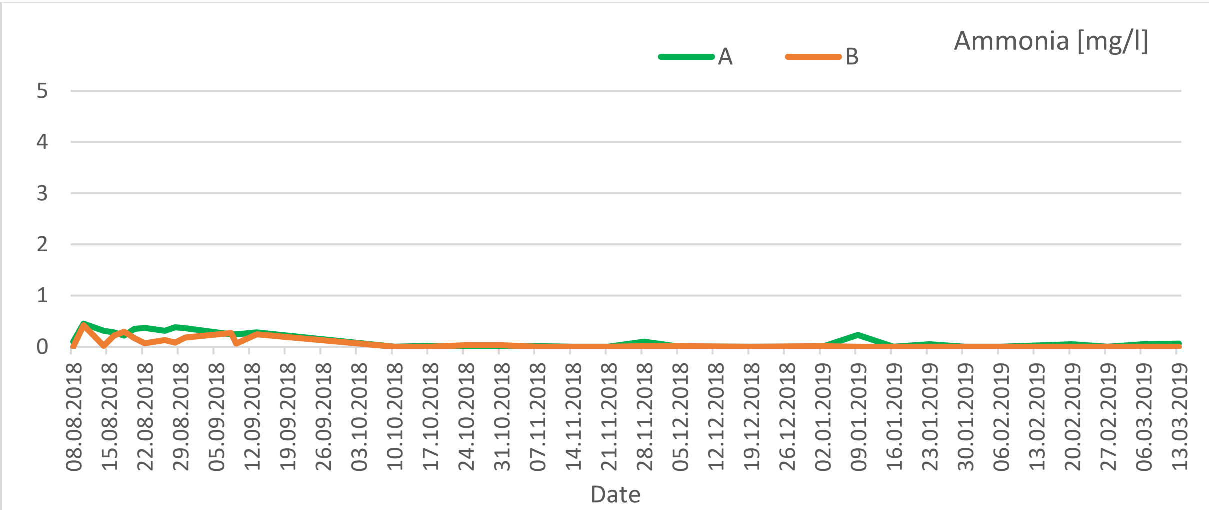


Fig. 14 Ammonia in the 2nd trial

To mitigate the possible occurrence of any algae in the system – which are favorable for shrimps, but may damage a RAS system, the phosphate and silicate absorbent bed was exchanged twice during the first trial, and three times during the second trial. Replacement of the adsorbent deposit decreased the phosphate and silicate concentration by 15-25%. There was no algae growth noticed during the trials.

Tab. 6 Nutrient concentration in farming water (mean±standard deviation)

	NH_4^+(mg/l)		NO_2^-(mg/l)		NO_3^-(mg/l)		PO_4^{3-}(mg/l)		SiO_2 (mg/l)	
	RAS A	RAS B	RAS A	RAS B	RAS A	RAS B	RAS A	RAS B	RAS A	RAS B
Trial 1	0.1±0.1	0.2±0.1	0.2±0.2	0.3±0.2	17.0±16.5	16.7±9.2	2.7±1.3	3.4±1.3	7.5±3.4	8.4±4.1
Trial 2	0.1±0.1	0.1±0.1	0.2±0.3	0.2±0.3	17.8±17.1	23.3±23.6	1.3±0.7	1.1±0.6	10.4±4.6	8.5±4.3

Feeding

PLs in the first trial consumed dry feed from the beginning. In the second trial, shrimps were much smaller so during nursery period they were fed with brine shrimp larvae. After acclimation, when the automatic feeders were used, daily dose for a tank was 1-31 g per day. The dose was calculated based on a shrimp number in the tank (counted directly or estimated from the mortality) and average shrimp mass (2.2.3.).

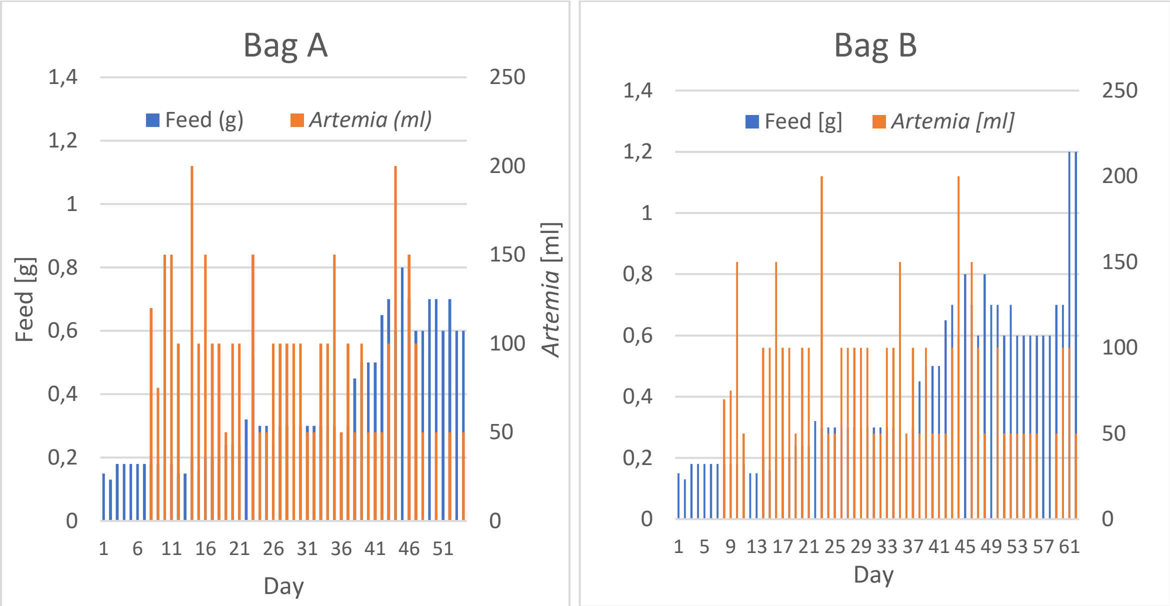


Fig. 15 Proportion of feed and *Artemia salina* during nursery period in the second trial

2.2.2. Survival

After 18 and 32 weeks of culture during trial 1 and trial 2 the survival of shrimps was 30 and 40% respectively. High mortality was not caused by natural causes, but rather by cannibalism. In addition, during the trial 1 several times there was a system failure, resulting in the death of a large number of shrimps.

2.2.3. Shrimp health status

<u>Trial 1</u>

As much as 86% of shrimps from trail 1 were characterized by mechanical damage to appendages such as antennas, antennules, rostrum, pereopods (walking legs) and pleopods (swimming legs), telson and uropods. Most often, antennas and antennule were damaged - this was noted in 41% of the studied shrimps. They might have appeared as a result of conspecific aggressive behavior. The least affected were the antennas - this was registered only in one individual (Fig. 16).

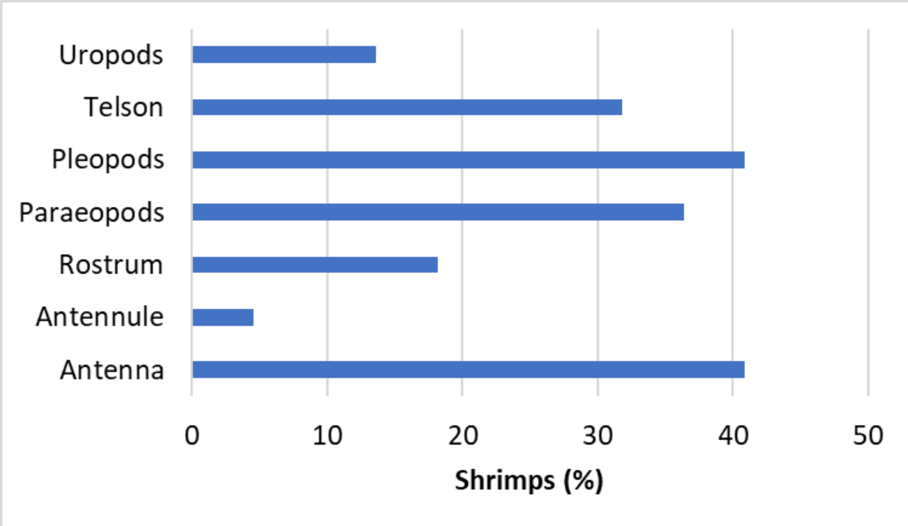


Fig. 16. Percentage of shrimps from trial 1 with bite marks on different parts of their body.

In 90% of cases, the lesion was colored black. This was the result of the process of melanization, i.e. the accumulation of blood cells at the site of infection and deposition of a black pigment (melanin). This type of melanization is known as point melanization (Fig. 17A). It can also appear as a result of other mechanical damage, for example related to rubbing against aquarium elements and other individuals, as well as bacterial infections not directly related to mechanical damage to the tissue. There were 25 such changes, which occurred 68% of analyzed shrimps. In 45% of shrimps there was also another type of melanization, so-called oblong (Fig. 17B). It was observed in the segmented part, usually at the joint of the ventral segments. In 18% of shrimps, within the ventral segments, thickening of the connective tissue was also observed. They are also likely to result from mechanical damage.

A B

Fig. 17. Different types of melanization observed in analyzed shrimps: A – point, B – oblong (Photo credit: Justyna Świeżak).

Fungal infections occurred in 59% of shrimps. They were in the form of white spots occurring singly or in clusters (Fig. 18), mainly on the inner side of the carapace, on paraeopods and uropods, less frequently seen on antennas, antennules and rostrum. In one shrimp, a large granulocytoma was observed and changes having the nature of progressive necrosis. A single case of a parasite (probably a fluke) was also observed.

Pathological changes occurring in internal organs of shrimps were most often associated (32%) with point or extensive inflammation characterized by infiltration of hemocytes within the damaged tissue of various area (Fig. 19A). These changes were mainly observed in the connective tissue of the digestive system, and in two cases in the muscles surrounding the internal organs. In the digestive system of 23% of the shrimps, the abnormalities consisting of atrophy (retardation) of digestive vesicles and hepatopancreas were also observed (Fig. 19B).

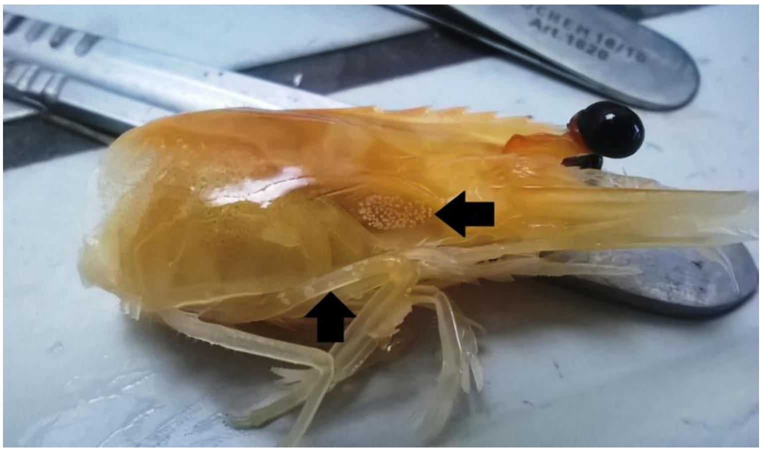


Fig. 18. Fungal infections (black arrows) on the paraeopods and carapax (photo credit: Justyna Świeżak).

A B

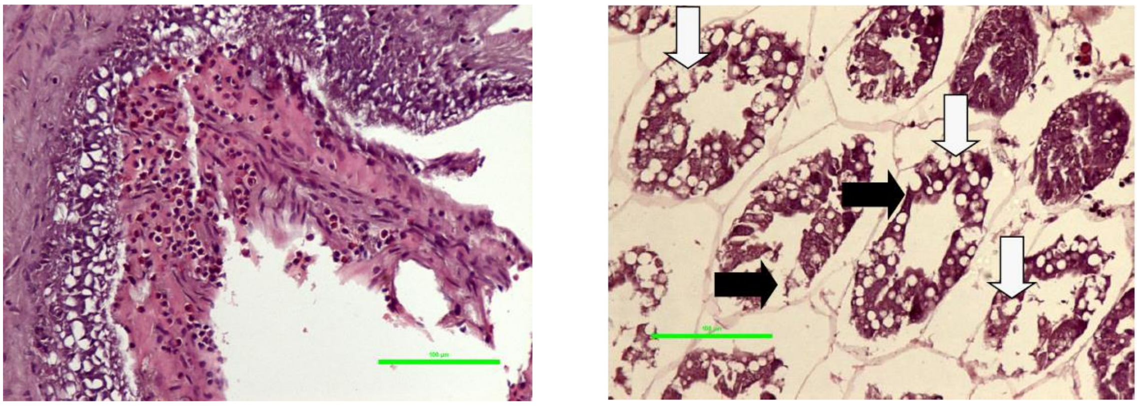


Fig. 19. Inflammation and fibrosis in the digestive tract (A) and section through the hepatopancreas (present fatty droplets - white arrows and bubble atrophy - black arrows). H & E dyeing (photo credit: Katarzyna Smolarz).

A B

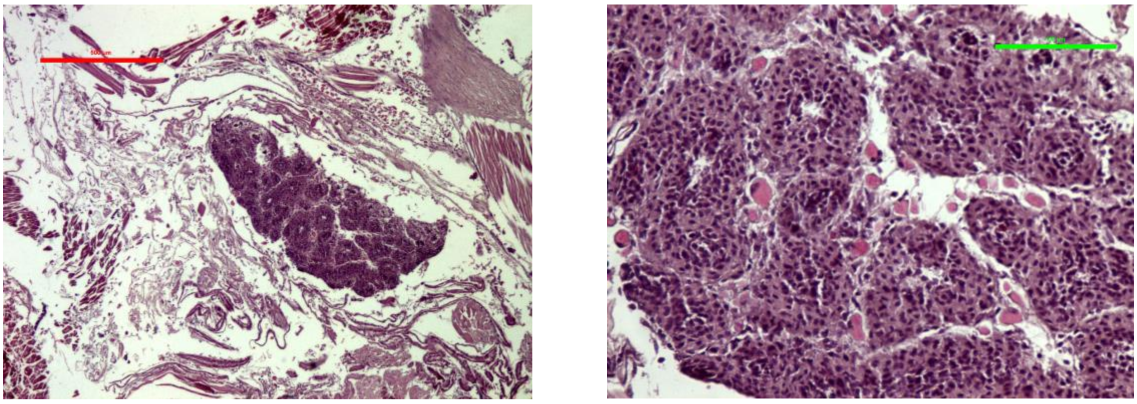


Fig. 20. Cross-section of the *L. vannamei* lymphoid organ (A), abnormal cell mass, spheroids are characterized by the disappearance of central lumen and basophilic cytoplasm (B). H & E dyeing (photo credit: Katarzyna Smolarz).

Trial 2

Among denoted external lesions in shrimps from trial 2 the most frequent (> 20%) were injuries of eyes, antenna, walking legs, abdominal segments, uropod, and telson. The least affected were smaller individuals, which seemed to be excluded from cannibalism. The most commonly devoured were antennae, very often reduced by more than 50% of their maximum length. Types of lesions observed in these body parts comprised: eye injuries (96%), necrosis and melanisation in the tips of walking legs, uropod, antenna (scales) and telson, as well as oblong melanisations on dorsal or side areas of the segments and protrusions on the joints of 3rd and 4th abdominal segments. Less frequently observed were lesions on antennule, swimmerets, rostrum and mouthparts (Fig. 21, Fig. 22). In 27 out of 28 specimens decolorization areas in the eyes were observed. The fading color is usually associated with mechanical abrasion that may lead to subsequent development of bacterial and viral infections (Smith, 2000). However, no signs of fungal diseases were found.

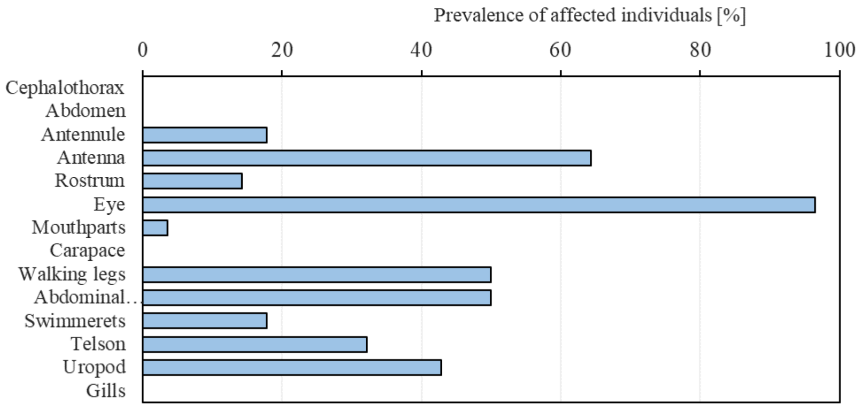


Fig. 21. Proportion of shrimps from trial 2 with external lesions of different body parts (n = 28).

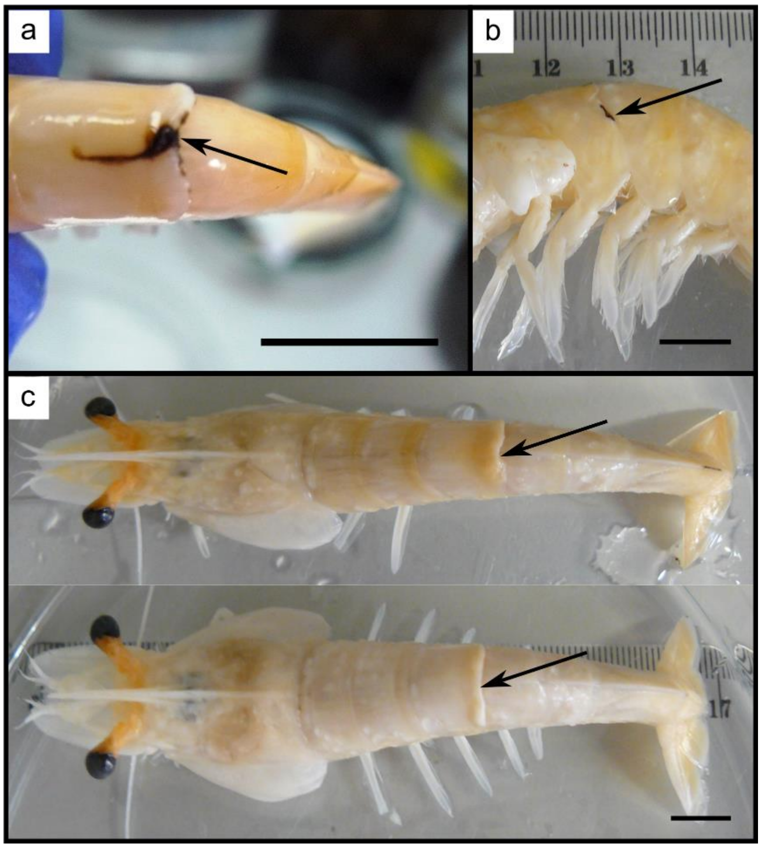


Fig. 22. External lesions found in shrimps from trial 2. Indicated by arrows: extensive melanisations on the top (a) and side (b) of abdominal segment and protrusions on the joint of 3rd and 4th abdominal segments of two exemplary specimens (c). All scale bars refer to 10 mm length (Photo credit: J. Świeżak).

Microscopic analyses showed that no major histological alterations in the hepathopancreas of *L. vannamei* were found what confirms macroscopical diagnosis. The only observed histopathologies in hepatopancreas included the enlargement of E- and F- cells together with decreased lumen area in various tubules (Fig. 23 A-C). They were found in 3 shrimps out of 28 analysed. Changed cells were often surrounded by haemocytic infiltration suggesting the ongoing inflammations and possible active transformation of E-cells into other, possibly dying, cell types (Fig. xxxD). Enlargement of B- and R- cells was also observed, each observed in one specimen and accompanied by decreased or no lumen space (Fig. 23E). In one case haemocytic infiltration was found suggesting the presence of local inflammation, but no other histological lesions were observed. Finally, in one case encapsulated parasite was found surrounded by heavy haemocytic infiltration (Fig 23F).

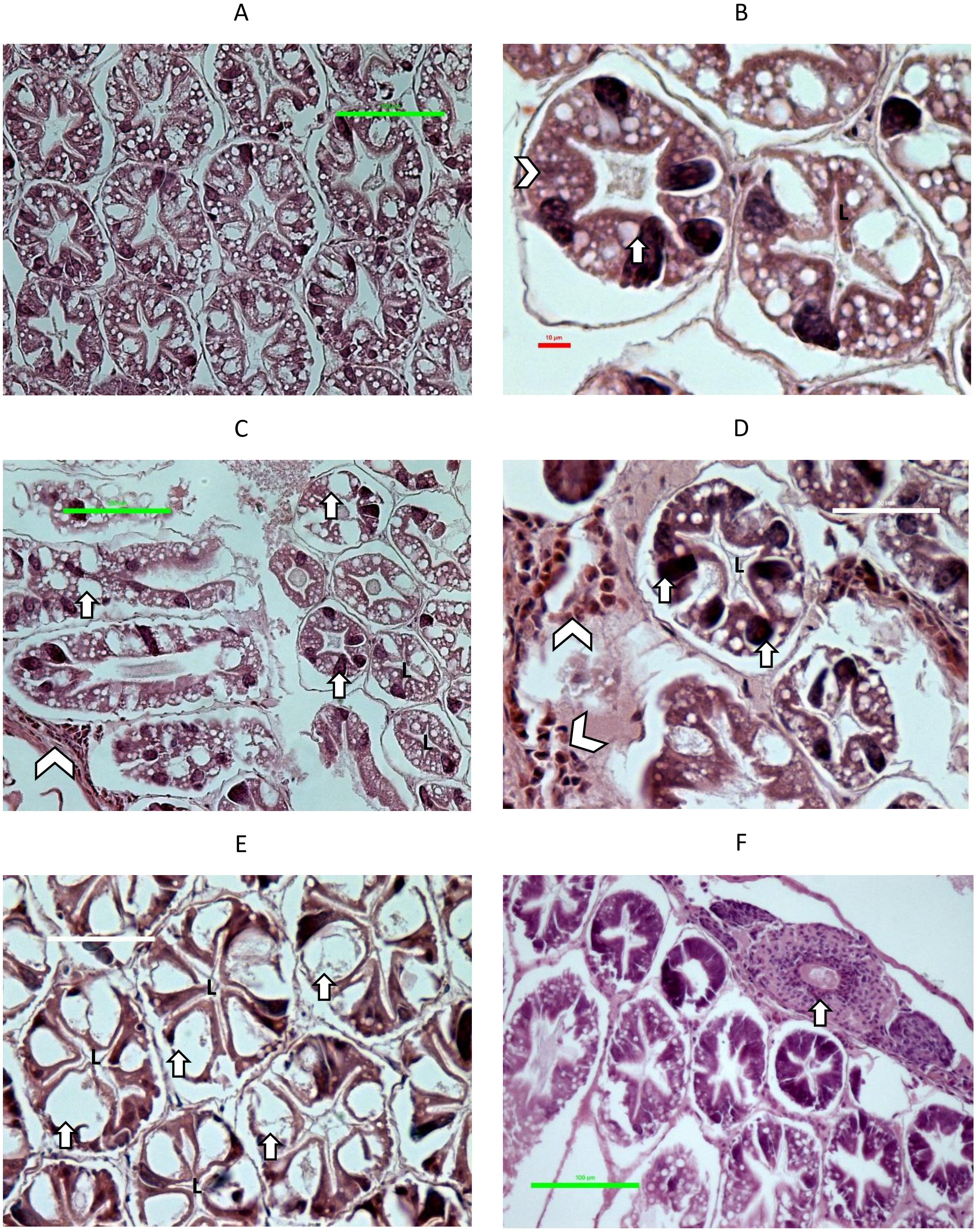


Fig. 23. Cross sections through the hepatopancreas (HP) of analysed shrimps. Normal HP tubules (A), enlargement of F- and E- cells (arrow and chevron, respectively) and decreased lumen space (B), enlargement of various cell types together with local haemocytic infiltration (arrows and chevrons, respectively) (C and D), enlargement of B-cells (arrows), note no lumen space in hepatopancreas tubules (E), encapsulated body, possibly a parasite, and hemocytic infiltration around the capsule (F). L – lumen, H&E staining (photo credit: K. Smolarz).

The anatomical and morphological changes that indicate a poor health condition, such a carapace erosion, gill defects, skin changes, decolourisation, inflammation, fungal infections, necrosis and eye lesions were observed in *L. vannamei* from trial 1 and 2. However, most of these changes were observed in single individuals (except for fungal infection and inflammation). Therefore, it was concluded that analyzed shrimps were in a relative good shape, healthy and fed properly.

2.2.4. Growth rate of cultured *L. vannamei*

<u>Trial 1</u>

The median length of shrimps during the 13 weeks of culture during trial 1 increased from 47 to 114 mm (Fig. 24 A), while the fresh mass from 0.567 to 9.841 g (Fig. 24 B). This means more than a 17-fold increase in mass at a 2.4-fold increase in length.

A B

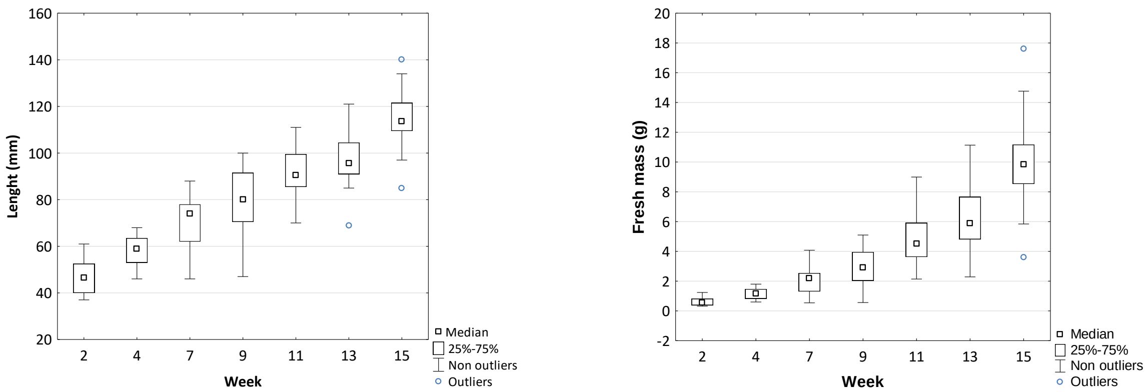


Fig. 24. Changes in length (A) and fresh mass (B) of *L. vannamei* in subsequent weeks of breeding during trail 1 (for each week n=20).

The largest mass gain (52% per week) occurred in the smallest shrimps, between weeks 2nd and 4th (Fig. 25). Later, the weight gain ranged from 16% per week (between week 11th and 13th) to 33% per week (between week 13th and 15th).

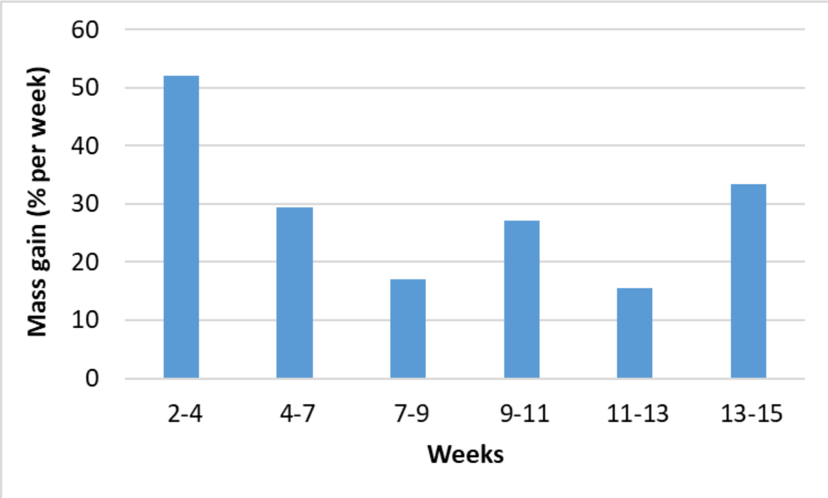


Fig. 25. Weight gain between successive weeks of breeding during trial 1 (calculated on the basis of median mass).

Fresh mass of analyzed shrimps from trial 1 (n=140) was significantly (R^2 = 0.99, $p < 0.05$) correlated with their length (Fig. 26).

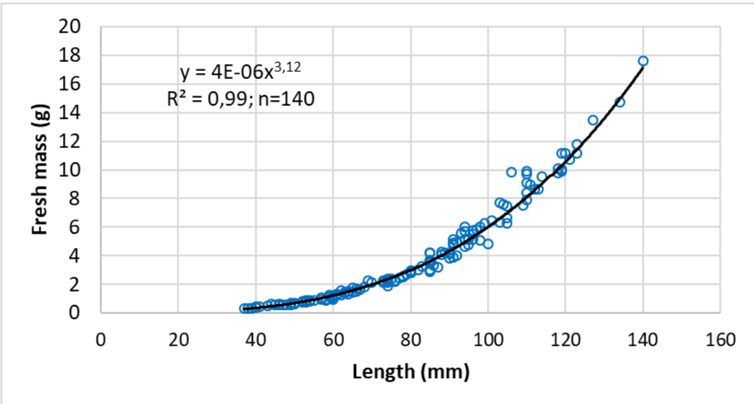


Fig. 26. Relationship between length and fresh mass of *L. vannamei* cultured during trial 1.

<u>Trial 2</u>

The median length of shrimps during the 21 weeks (between week 8th and 29th) of culture during trial 2 increased from 18 to 100 mm (Fig. 27A), while the fresh mass from 0.018 to 5.360 g (Fig. 27B). This means almost a 300-fold increase in mass at a 5.7-fold increase in length.

A B

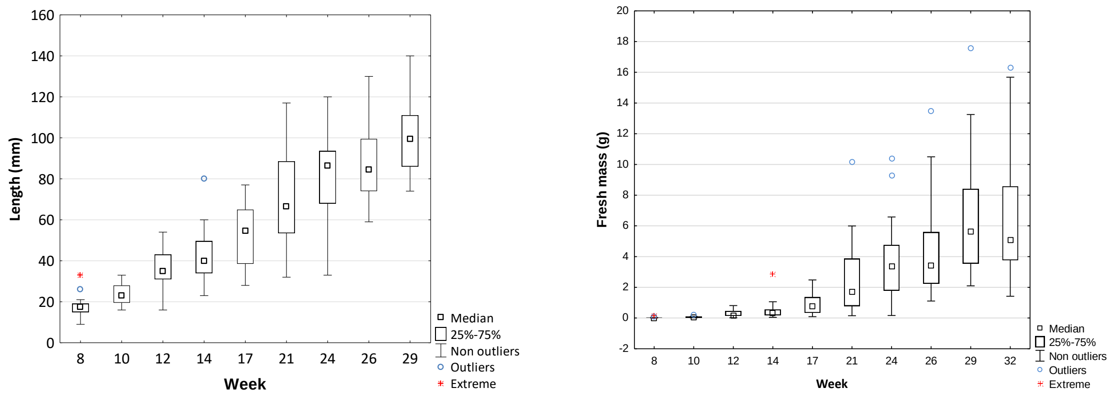


Fig. 27. Changes in length (A) and fresh mass (B) of *L. vannamei* during trail 2 of experimental culture (for each week n=20).

The largest mass gain (115 and 100% per week) occurred in the smallest shrimps, between weeks 8th and 10th and 10th and 12th (Fig. 28). Later, the weight gain varied from 1% per week (between week 24th and 26th) to 54% per week (between week 14th and 17th).

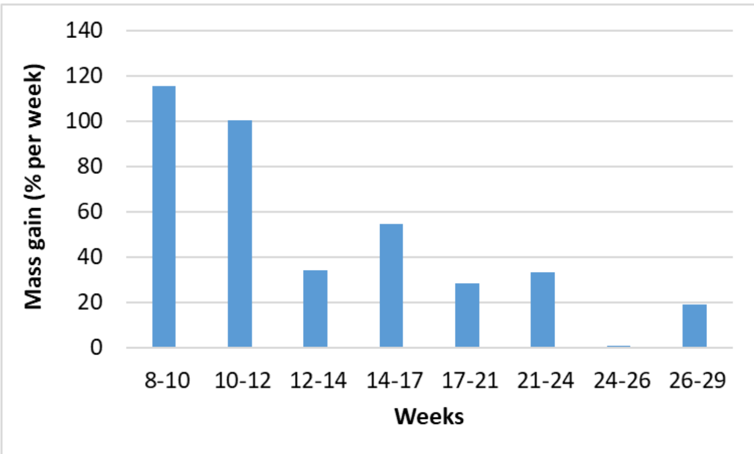


Fig. 28. Weight gain between successive weeks of breeding during trial 2 (calculated on the basis of median mass).

Fresh mass of shrimps from trial 2 (n=183) was significantly (R^2 = 0.99, $p < 0.05$) correlated with their length (Fig. 29).

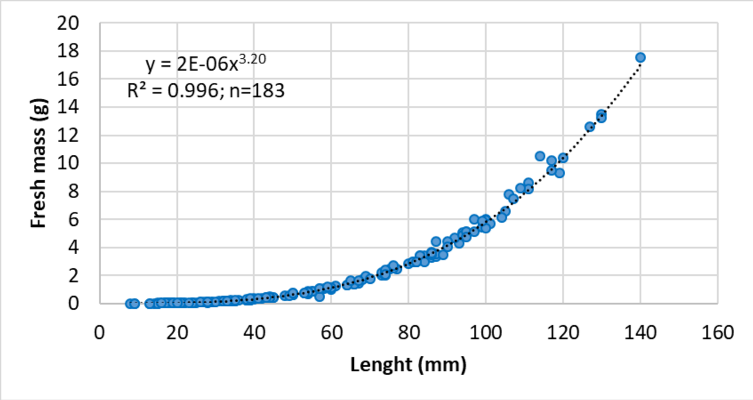


Fig. 29. Relationship between length and fresh mass of *L. vannamei* cultured during trial 2.

Trial 2 lasted for 14 weeks longer, which was the time needed for shrimps to reach a size such as the initial size in the trial 1. The length-fresh mass relationships for 13 weeks for shrimps cultured in both trials (week 2-15 for trial 1 and week 14-29 for trial 2) were similar what shows a similar rate in growth. In addition, value of the power (b > 3) in the relationship between the fresh mass and length of shrimps from trial 1 and 2 may indicate good condition of cultured shrimps.

2.2.5. Energy value and organic matter content

Trial 1

Information on specimens from trial 1 used for analyses of energy value and organic matter content is given in the Table 7. The proportion of abdominal muscle mass in the total mass of the shrimp significantly increased ($p < 0.05$, $R^2 = 0.37$) with the length of the individual according to the formula $y = 1.462 x + 28.702$.

Table. 7. Characteristic of *L. vannamei* from trial 1 used for analyses of energy value. Values are given as the mean ± SD.

Parameter	Length class (cm)				
	8.9-9.9	**10.1-10.9.0**	**11.0-11.9**	**12.0-12.9**	**13.0-13.9**
Number of shrimps	5	5	5	5	5
Length (cm)	9.0 ± 0.2	10.2 ± 0.2	11.2 ± 0.2	12.2 ± 0.2	13.1 ± 0.2
Fresh mass (g)	3.9 ± 0.3	6.2 ± 0.6	8.0 ± 1.0	10.4 ± 0.5	13.2 ± 0.9
Abdominal muscle (% of fresh mass)	40.3 ± 2.2	45.2 ± 2.9	45.3 ± 3.3	48.0 ± 3.1	46.1 ± 1.2

Mean energy value of abdominal muscle of *L. vannamei* from trial 1 varied from 17.4 to 22.0 J mg^{-1} DW (mean value 19.8 ± 1.0 J mg^{-1} DW, n=25) and did not differ ($p > 0.05$) between specimens from subsequent length classes (Fig. 30).

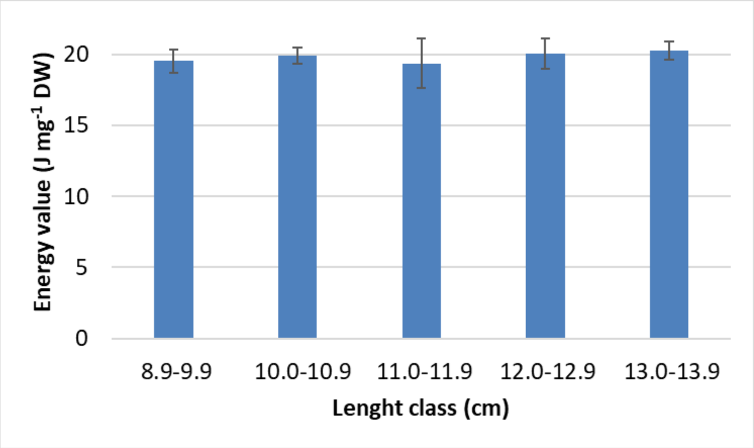


Fig. 30. Mean (± SD) energy value of abdominal muscle of *L. vannamei* from trial 1, in subsequent length classes.

Mean content of organic matter in abdominal muscle of *L. vannamei* from trial 1 varied from 92.4 to 95.3 % DW (mean 93.5 ± 0.8 %DW, n=25) and did not differ ($p > 0.05$) between specimens from subsequent length classes (Fig. 31).

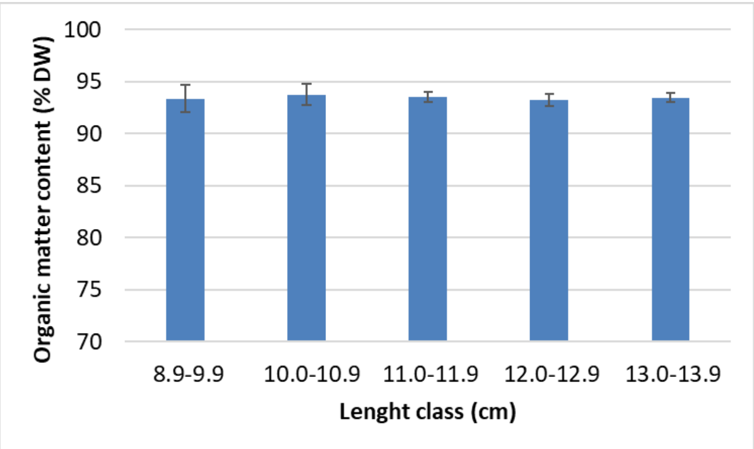


Fig. 31. Mean (± SD) organic matter content in abdominal muscle of *L. vannamei* from trial 1, in subsequent length classes.

Trial 2

Information on specimens from trial 2 used for analyses of energy value and organic matter content is given in the Table 8. Both, the length and fresh mass of shrimps from subsequent length classes differed significantly ($p < 0.05$). Also, the proportion of abdominal muscle mass in the total weight of the shrimp and water content in abdominal muscle differed significantly ($p < 0.05$) in subsequent length classes.

Table. 8. Characteristic of *L. vannamei* from trial 2 used for analyses of energy value. Values are given as the mean ± SD. Mean values marked with different letters are statistically significant ($p < 0.05$)

Parameter	**Length class (cm)**		
	8.9-10.0	**11.1-12.0**	**13.1-14.0**
Number of shrimps	6	6	6
Length (cm)	9.5 ± 0.4[a]	11.5 ± 0.2[b]	13.3 ± 0.2[c]
Fresh mass (g)	4.58 ± 0.63[a]	8.04 ± 0.40[b]	13.85 ± 0.83[c]
Abdominal muscle (% of fresh mass)	45.0 ± 0.8[a]	47.8 ± 1.2[b]	48.6 ± 1.7[c]
Water content in abdominal muscle (%)	74.1 ± 0.7[a]	75.6 ± 0.5[b]	76.8 ± 0.1[c]

The proportion of abdominal muscle mass in the total weight of the shrimp significantly increased ($p < 0.05$, $R^2 = 0.62$) with the length of the individual according to the formula $y = 0.974\,x + 36.042$. Also, the water content in the abdominal muscle significantly increased ($p < 0.05$, $R^2 = 0.58$) along with the length of the individual according to the formula $y = 0.499\,x + 69.581$.

Mean energy value of abdominal muscle of *L. vannamei* from trial 2 varied from 17.0 to 19.4 (mean 18.0 ± 0.6 J mg^{-1} DW, n=18) and did not differ between specimens from three length classes (Fig. 32).

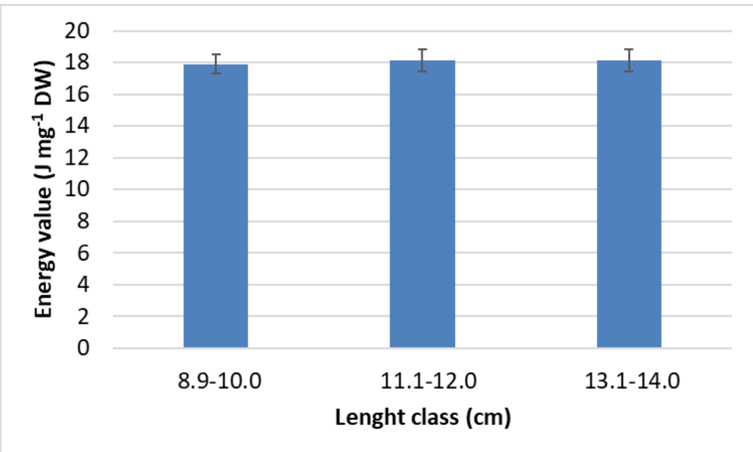


Fig. 32. Mean (± SD) energy value of abdominal muscle of *L. vannamei* from trial 2, in subsequent length classes.

Mean content of organic matter in abdominal muscle of *L. vannamei* from trial 2 varied from 93.4 to 94.7 %DW (mean 94.0 ± 0.3 %DW, n=18) and did not differ ($p > 0.05$) between specimens from three length classes (Fig. 33). The content of organic matter decreased significantly ($p < 0.05$, $R^2 = 0.28$) along with the dry mass of the abdominal muscle, according to the equation $y = -0.374 x + 94.379$.

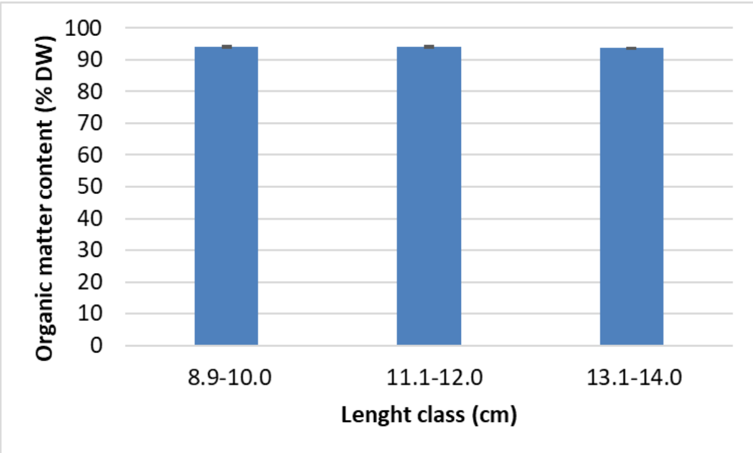


Fig. 33. Mean (± SD) organic matter content in abdominal muscle of *L. vannamei* from trial 2, in subsequent length classes.

The energy value of shrimps from trial 2 was smaller than those from trial 1, while the content of organic matter was similar. In the analyzed shrimps, characterized by a total length in the range of 8.9-13.5 cm, both the energy value and organic matter content were similar.

2.2.6. Carbon, hydrogen and nitrogen content

Trial 1

Content of carbon in abdominal muscle of *L. vannamei* from trial 1 varied between 43.0 and 44.8 %DW (mean 44.2 ± 0.6 %DW, n=25), hydrogen content from 6.7 to 7.7 %DW (mean 7.1 ± 0.3 %DW, n=25) and nitrogen from 13.1 to 13.9 %DW (mean 13.6 ± 0.3 %DW, n=25). Biggest shrimps were characterized by higher contents of carbon, hydrogen and nitrogen compared to smallest one (Fig. 34). There was significant, positive correlation ($p < 0.05$) between content of carbon, hydrogen and nitrogen and dry mass of the abdominal muscle (Fig. 35).

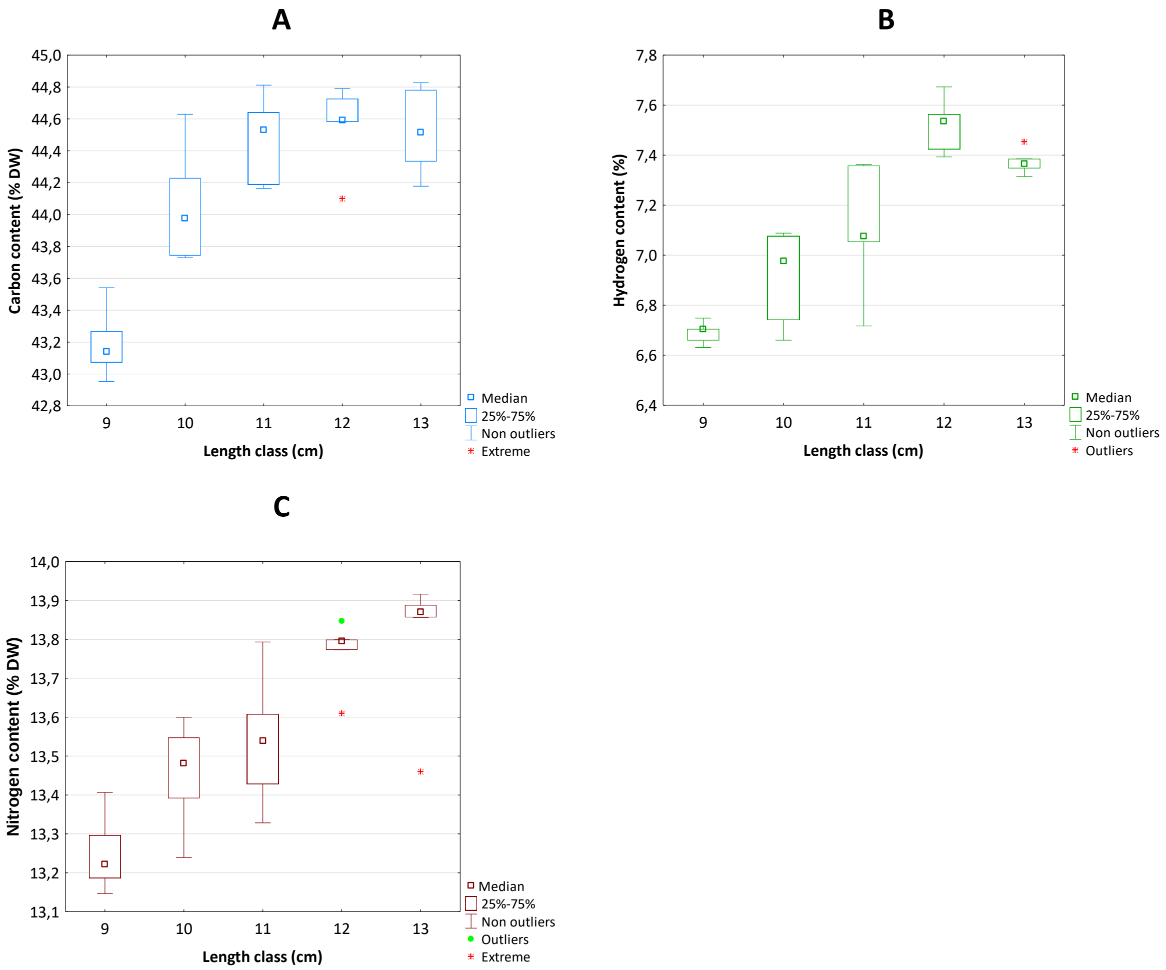
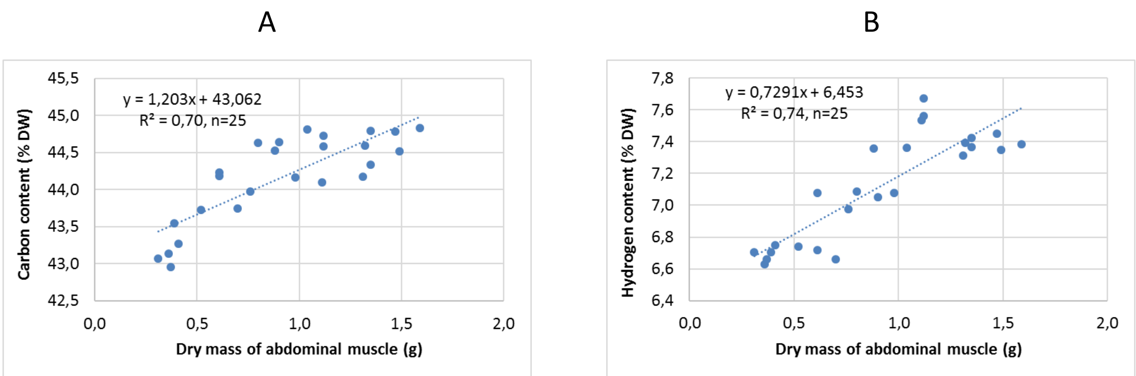


Fig. 34. Content of carbon (A), hydrogen (B) and nitrogen (C) in the abdominal muscle of *L. vannamei* from trial 1, in subsequent length classes.



C

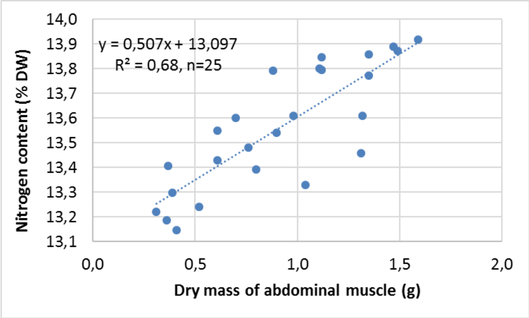
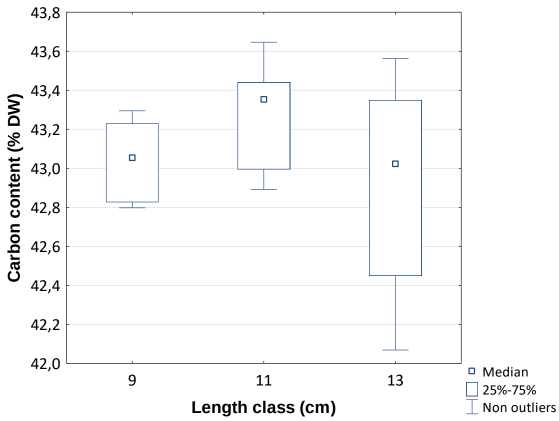


Fig. 35. Relationship between carbon (A), hydrogen (B) and nitrogen (C) content and dry mass of abdominal muscle of *L. vannamei* from trial 1.

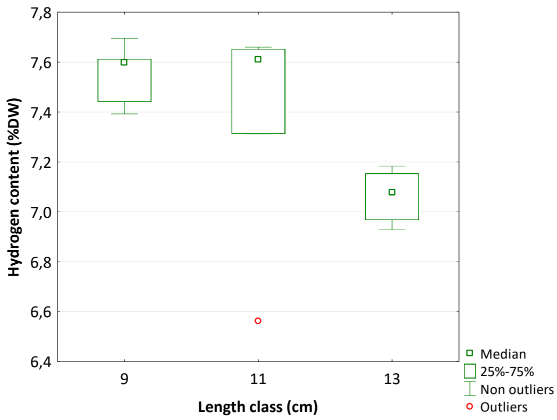
Trial 2

Mean content of carbon in abdominal muscle of *L. vannamei* from trial 2 was 43.1 ± 0.4 %DW (n=18) and did not differ ($p > 0.05$) between specimens from three length classes (Fig. 36A). The hydrogen content ranged from 6.6 to 7.7 %DW (mean 7.3 ± 0.3 %DW, n=18), whereas nitrogen from 13.2 to 13.8 %DW (mean 13.4 ± 0.2 %DW, n=18). In the biggest shrimps hydrogen was significantly ($p < 0.05$) lower, whereas nitrogen content significantly ($p < 0.05$) higher than in smaller shrimps (Fig. 36B and C). The content of hydrogen decreased significantly ($p < 0.05$, $R^2 = 0.34$), according to the equation $y = -0.406 x + 7.759$, whereas the content of nitrogen increased significantly ($p < 0.05$, $R^2 = 0.430$) along with the dry mass of the abdominal muscle, according to the equation $y = 0.219 x + 13.218$. The C : N ratio decreased significantly ($p < 0.05$, $R^2 = 0.35$) along with the dry mass of the abdominal muscle, according to the equation $y = -0.058 x + 3.264$.

A



B



C

D

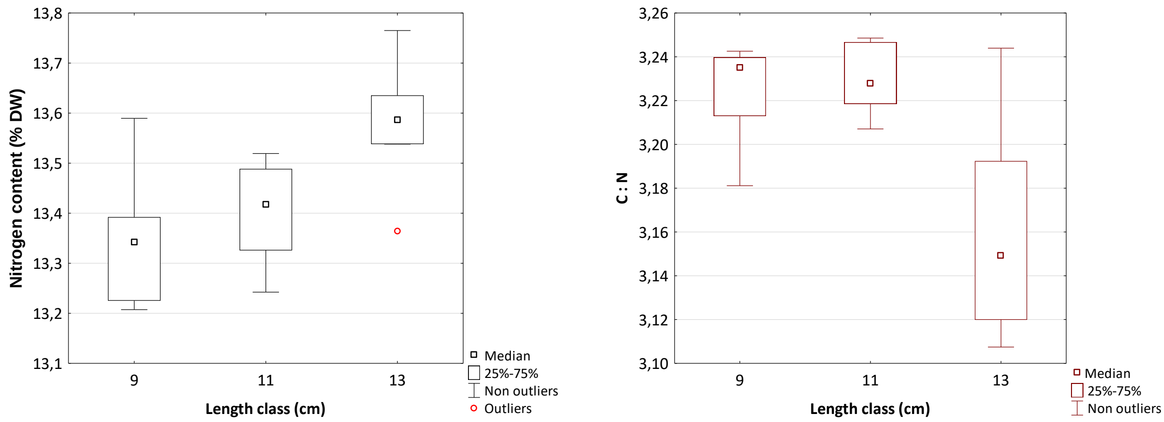


Fig. 36. Content of carbon (A), hydrogen (B), nitrogen (C) and the ratio of carbon to nitrogen in the abdominal muscle of *L. vannamei* from trial 2, in subsequent length classes.

In the case of shrimps from trial 1, larger individuals (> 12.1 cm) were characterized by a higher content of carbon, hydrogen and nitrogen than smaller individuals (<9.9 cm). This may indicate their better nutritional value. In the case of shrimps from trial 2, larger individuals were characterized by a lower C: N ratio, which is an indicator of lipid content, and by a higher nitrogen content, which is an indicator of protein content. On the other hand, they also contained more water and less organic matter than smaller shrimps.

2.2.7. Organoleptic properties

Trial 1

The first batch of shrimps (from trial 1) was delivered as a whole fresh shrimps with the mass 75.19 psc/kg (Fig. 37). The average mass of delivered *L. vannamei* was lower than the average mass of whiteleg shrimps placed on the market.

Fig. 37. The analyzed batch of shrimps from trial 1 (Photo credit: Olga Szulecka).

The results of the morphometric analyzes are presented in the Table 9, whereas the results of the sensory tests in the figures 38-46.

Table 9. Average mass and length of analyzed shrimps from batch from trial 1.

Parameter	Lenght [cm]	Mass [g]
Average ± SD	13.3 ± 0.75	16.42 ± 2.85
X min	12.00	11.25
X max	15.00	24.07
The total number of the shrimps [pcs]	25	
The number of the shrimps in kg [psc/kg]	75.19	

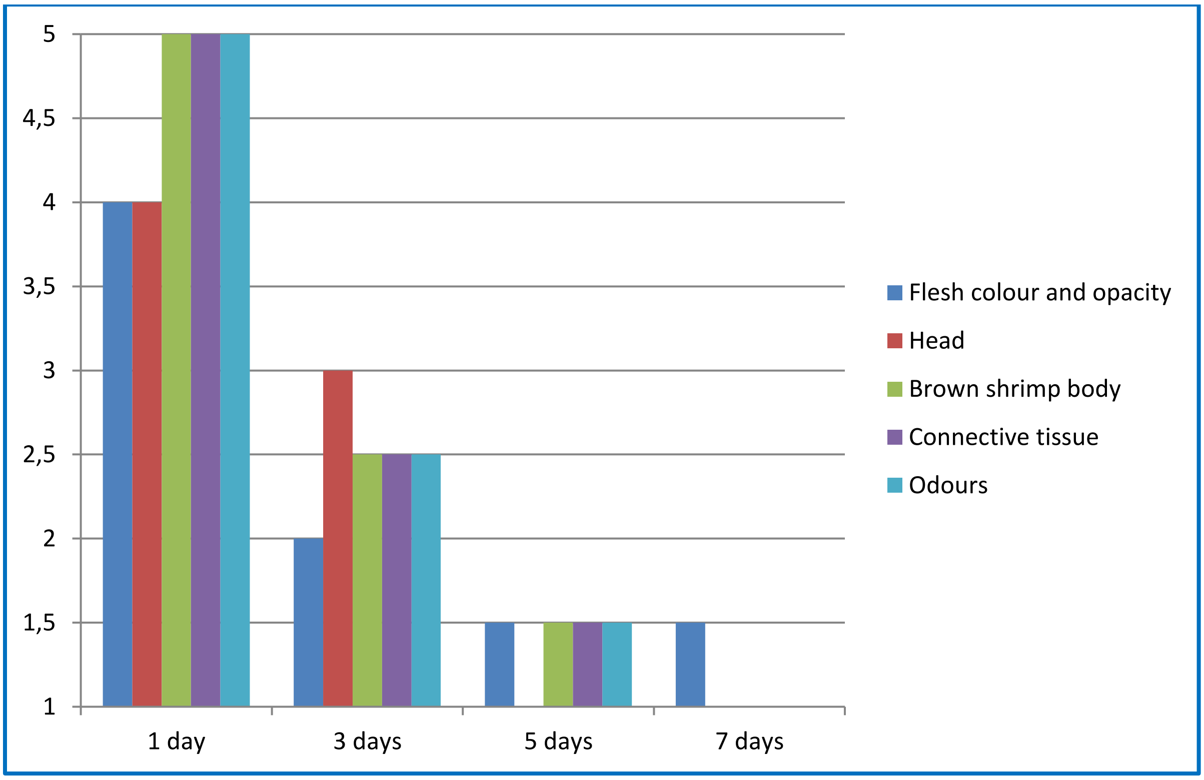


Fig. 38. Sensory assessment of fresh (raw) shrimps from trial 1 after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 39. Fresh (raw) shrimps from batch from trial 1 after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

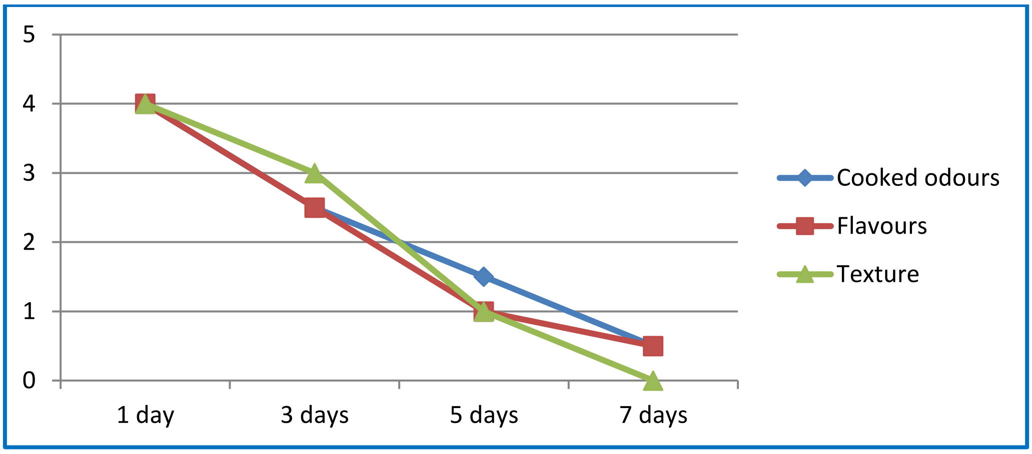


Fig. 40. Sensory assessment of fresh (cooked) shrimps from batch from trial 1 after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 41. Fresh (cooked) shrimps from batch from trial 1 after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The half of the analyzed batch was frozen and then defrosted. The defrosted batch is presented in the Fig. 42.

Fig. 42. The analyzed batch of frozen shrimps from trial 1 after defrosting (Photo credit: Olga Szulecka).

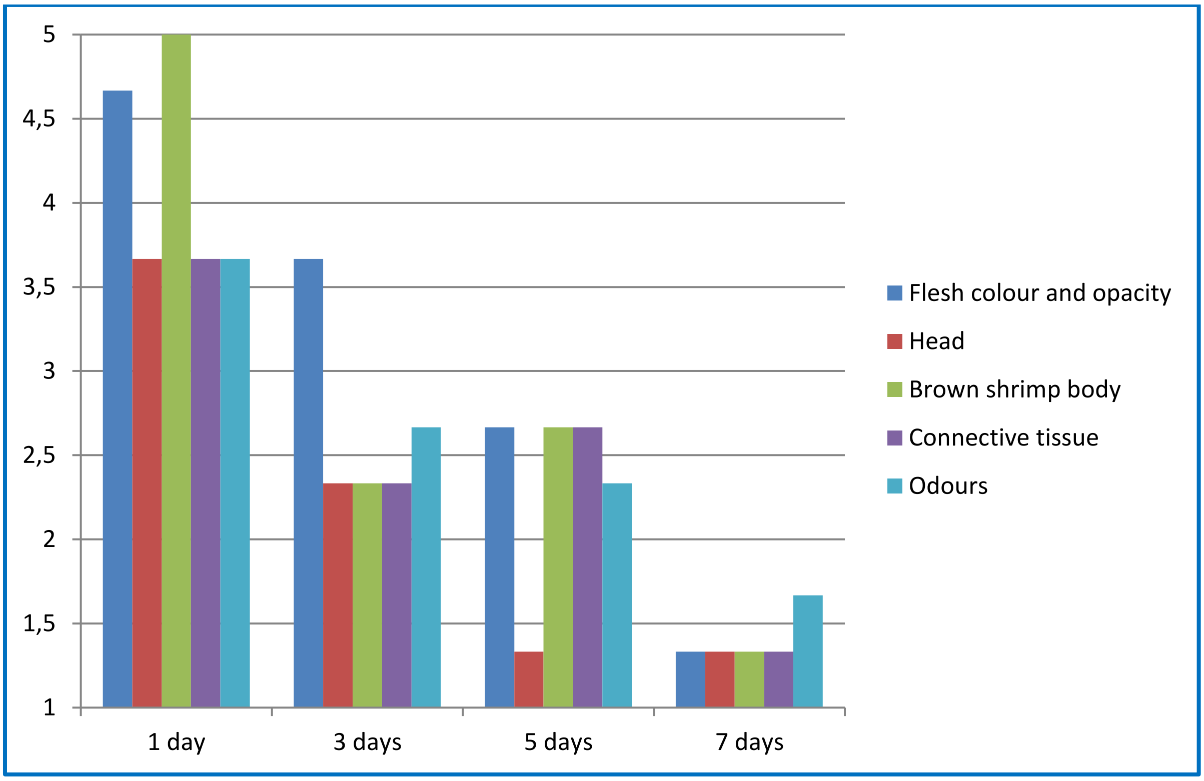


Fig. 43. Sensory assessment of frozen (raw) shrimps from trial 1 after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

A

B

Fig. 44. Frozen (raw) shrimps from batch from trial 1 after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

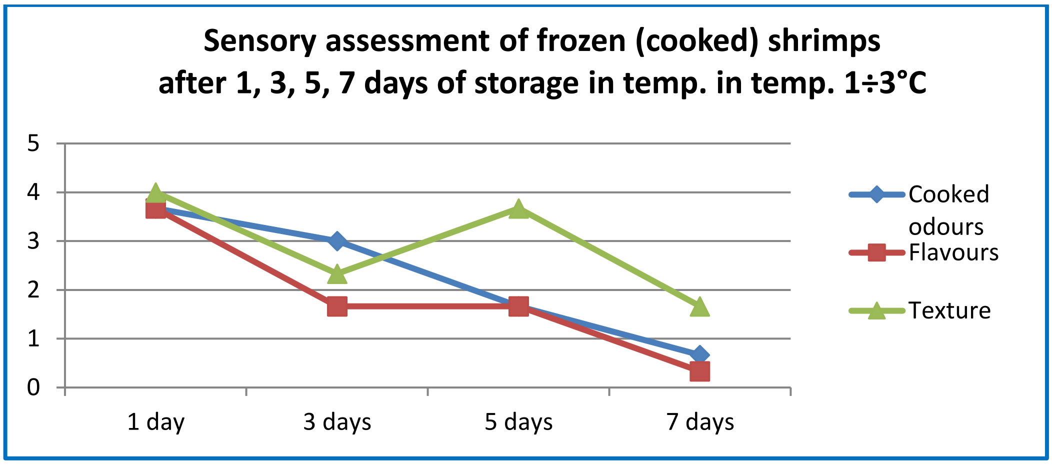


Fig. 45. Sensory assessment of frozen (cooked) batch of shrimps from trial 1 after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 46. Frozen (cooked) shrimps from batch from trial 1 after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

<u>Trial 2</u>

The batches of shrimps from trial 2 were taken separately from RAS-1 and RAS-2 and also analyzed separately during the week of storage. The results of the morphometric analyzes of shrimps from RAS-1 (Fig. 47) are presented in the Table 10, whereas of sensory tests in the figures 48-51.

Fig. 47. The analyzed batch of shrimps from batch from trial 2, taken from RAS-1 (Photo credit: Olga Szulecka).

The half of analyzed batch of shrimps from RAS-1 was headed and the average mass of the headed part of batch amounted 8.18 ± 1.36 g and the average yield of it was 62.81 ± 1.05%.

Table 10. Average mass and length of analyzed shrimps from batch from trial 2, taken from RAS-1.

Parameter	Lenght [cm]	Mass [g]
Average ±SD	11.69±1.24	10.93±3.28
X_{min}	9.50	5.08
X_{max}	13.50	16.50
The total number of the shrimps in analised batch [pcs]	16	
The number of the shrimps in kg [psc/kg]	91.51	

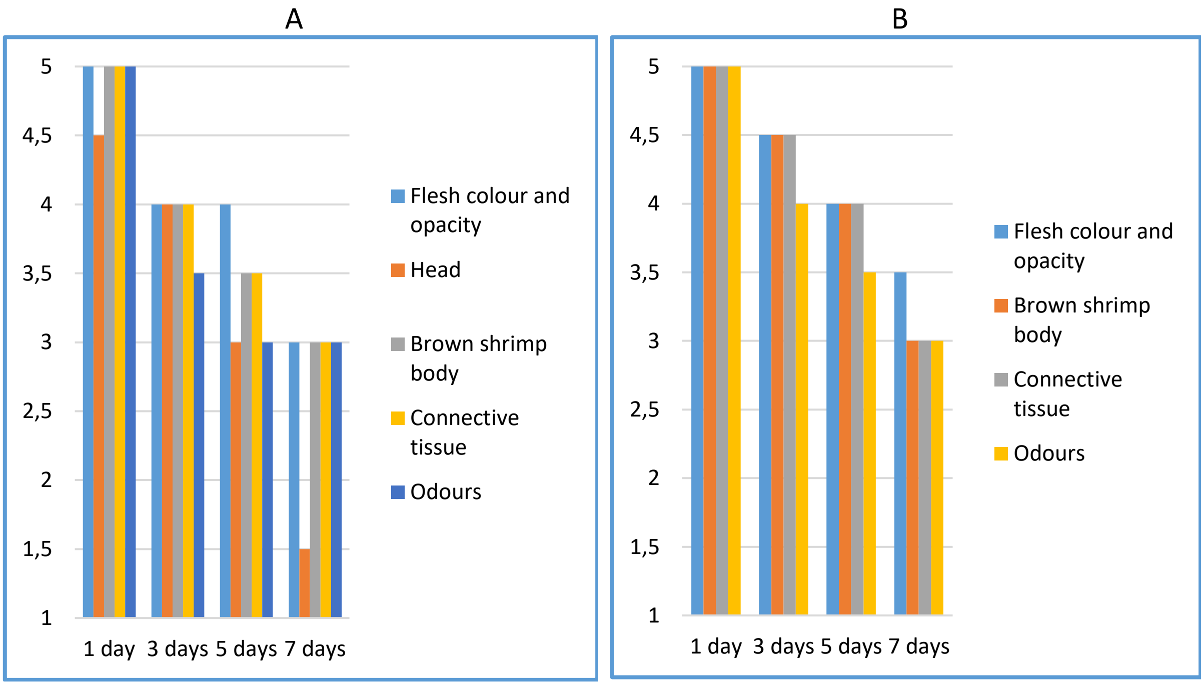


Fig. 48. Sensory assessment of fresh (raw) of whole (A) and headed (B) shrimps from RAS-1 (trial 2) after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 49. Fresh (raw), whole and headed shrimps from batch from RAS-1 (trial 2) after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

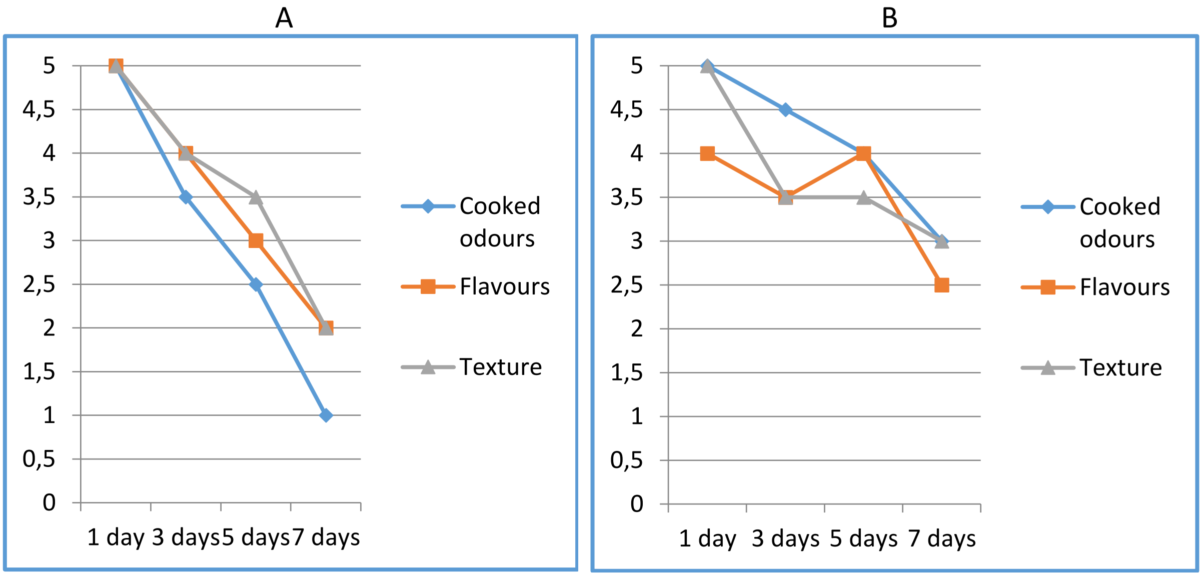


Fig. 50. Sensory assessment of fresh (cooked) whole (A) and headed (B) shrimps from RAS-1 (trial 2) after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 51. Fresh (cooked), whole and headed shrimps from batch from RAS-1 (trial 2) after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The batch of whole shrimps from RAS-2 (trial 2) were much smaller than those from RAS-1. The results of the morphometric analyzes of shrimps from RAS-2 (Fig. 52) are presented in the Table 11, whereas the results of the sensory tests in the figures 53-56.

Fig. 52. The analyzed batch of shrimps from batch from trial 2, taken from RAS-2 (Photo credit: Olga Szulecka).

The half of analyzed batch of shrimps was headed and the average mass of the headed part of batch was 4.65 ± 1.54 g and the average yield of it was 61.36 ± 2.05%.

Table 11. Average mass and length of analyzed shrimps from batch from trial 2, taken from RAS-2.

Parameter	**Lenght [cm]**	**Mass [g]**
Average ±SD	9.55±1.21	6.10±2.12
X_{min}	7.50	2.77
X_{max}	11.50	9.43
The total number of the shrimps in analised batch [pcs]	10	
The number of the shrimps in kg [psc/kg]	164.07	

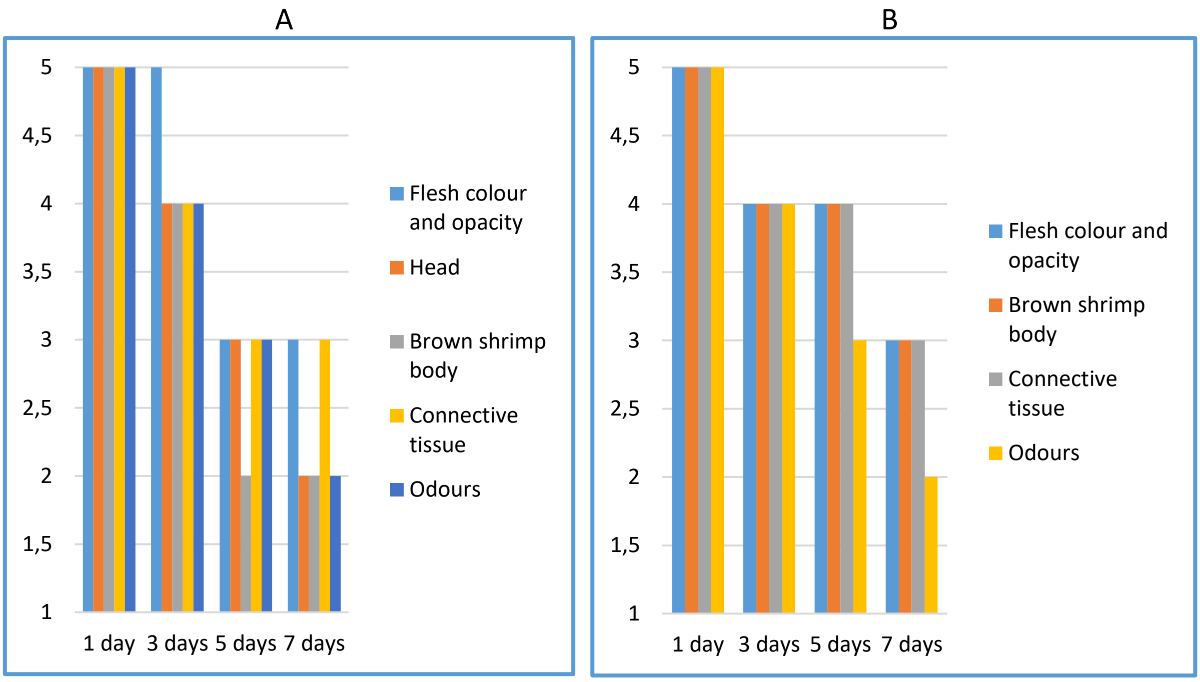


Fig. 53. Sensory assessment of fresh (raw) of whole (A) and headed (B) shrimps from RAS-2 (trial 2) after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 54. Fresh (raw), whole and headed shrimps from batch from RAS-2 (trial 2) after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

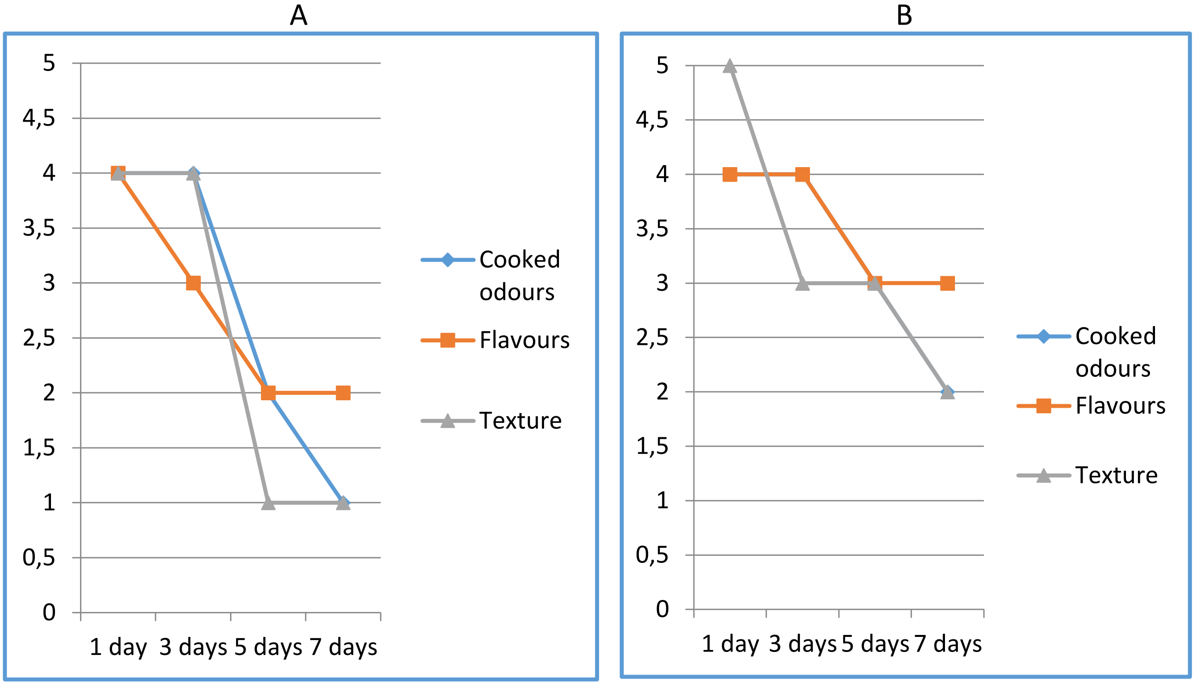


Fig. 55. Sensory assessment of fresh (cooked) whole (A) and headed (B) shrimps from RAS-2 (trial 2) after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 56. Fresh (cooked), whole and headed shrimps from batch from RAS-2 (trial 2) after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The results of the analyses of fresh shrimp batch from trial 1 showed that the delivered shrimps were characterized by fresh meat however without using and preservatives substances the quality of the shrimps (raw and cooked) has been significantly reduced during storage time. Freezing has reduced the speed of the negative quality changes of defrosted shrimps during cold storage in comparison to fresh shrimps and the final results of frozen raw shrimps were better than fresh raw ones. Also during the storage time the texture of frozen cooked meat obtained better scores than in the fresh cooked meat.

The results of analyzed shrimps from batch from trial 2, taken from RAS-1 showed that the quality of whole shrimps remained at the lower level for the period of week storage than of the headed ones. The whole shrimps have in the 7th day of storage blackish head which causes the lower taste and texture of meat at the end of storage. The results of analyzed shrimps from batch from trial 2, taken from RAS-1 also showed that the quality of whole shrimps remained at the lower level for the week storage period than the headed ones. The analyzed batch was too small to correctly prove that statement however the results presented in the graphs show the difference. The blackish head of whole shrimps in the 7th day of storage causes the lower taste and texture of meat at the end of storage.

3. Quality of shrimps imported to Polish market from world's farms and shrimps from RAS

3.1. Materials and methods

3.1.1. Analysed shrimps

Analyses were performed on four species of shrimps available on the Polish market, which were:

- Indian kidi (*Parapenaeopsis stylifera*) caught in the western Indian Ocean (FAO 51),
- red argentinian (*Pleoticus muelleri*) caught in the south-west Atlantic (FAO 41),
- gangs (*Penaeus monodon*) bred in India and Bangladesh,
- white (*Litopenaeus vannamei*) bred in India, Vietnam and Ecuador.

In addition white shrimp *L. vannamei* from three different RAS were studied:

- white (*Litopenaeus vannamei*) from experimental RAS culture in Poland (Pilot 2), from trial 1 and trial 2,
- white (*Litopenaeus vannamei*) from commercial farm located in Germany,
- white (*Litopenaeus vannamei*) from experimental RAS culture in Lithuania (Pilot 1).

Together with shrimps from RAS in Poland their feed used for breeding was studied.

3.1.2. Analyses of flesh quality

Chemical analysis of shrimps were conducted by NMFRI. They involved : 1) nutritional value, 2) levels of chemical contaminants which may occur in shrimps as a result of accumulation from an environment or feed: heavy metals- Hg, Pb, Cd, organochlorine pesticides- DDTs, polychlorinated biphenyls (six indicator congeners).

<u>Determination of fat content and fatty acids</u>

Samples of freeze-dried shrimp flesh and feed were homogenized in a dichloromethane: methanol (2:1,v:v) solution, from which total lipids were extracted. The extract was dried briefly under argon gas and residual lipid extract was trans-methylated with methanolic solution of potassium hydroxide, hydrochloric acid and isooctane. The methyl esters formed were separated in a gas chromatograph (Agilent Technologies 6890N GC) using an RT-2560 capillary column (length 100 m, internal diameter 0.25 mm and film thickness 0.2µm; Restek, USA) and a flame ionization detector. Individual FA methyl esters were identified and quantified in relation to known standards analyzed together with samples, using Agilent Technologies software (Chemstation).

<u>Determination of micro and macroelements</u>

Homogenized shrimp flesh samples and ground feed samples were wet digested with concentrated nitric acid in MD-2100 microwave oven. The final determinations were performed with

the atomic absorption method in a graphite furnace with a Perkin Elmer 4100 atomic absorption spectrometer with plasma excitation using a VISTAMPX emission spectrometer.
Mercury analysis was performed with flameless atomic absorption spectrometry using an Altec AMA-254 spectrophotometer. The measurement series were preceded by measuring the mercury concentration in reference material – SRM 1566b (oyster tissue).

Determination of vitamins

Homogenized, freeze-dried shrimp flesh samples and ground feed samples were saponificated and vitamins were extracted with hexane. Extracts were evaporated, and the residue was dissolved in methanol. Final determinations were performed with the HPLC technique. Vitamins A and E were determined by fluorescence and vitamin D_3 with a UV detector. The quantitation of vitamins A and E was performed based on the area of standard peaks, while vitamin D_3 was determined using vitamin D_2 as the internal standard. The internal standard was added to samples prior to saponification.

Determination of pesticides (HCHs, HCB, DDTs) and polychlorinated biphenyls (PCBs)

Homogenized, freeze-dried shrimp flesh samples and ground feed samples were extracted with n-hexane in a Soxtec Avanti apparatus (FOSS) for 4 h. An aliquot of lipid (0.5 g of oil) was dissolved in n-hexane in stoppered glass tubes and treated with a mixture of concentrated sulfuric acid and 30% fuming sulfuric acid at a ratio 1:1 v/v. It was shaken periodically for 3 h. After centrifugation samples were frozen and the upper solvent phase was collected. A sulfuric acid phase was defrosted and reextracted with hexane. Combined extracts were adjusted to a volume of 1 mL and analyzed using the GC/ECD technique. Quantitation was conducted based on the standard peak area. Results for PCBs were expressed as a sum of six congeners (IUPAC nos. 28, 52, 101, 138, 153 and 180) and pp-DDE, pp-DDD and pp-DDT were also summed and expressed as a sum of DDTs.

3.1.3. Organoleptic properties

Organoleptic analyses were performed using fresh and frozen (whole and headed) *L. vannamei* shrimps available on the Polish market (symbols: INA-18, INA-19, INA- 22, INA-23, INA-24) and frozen (whole and headed) *L. vannamei* shrimps obtained from Lithuanian partner (Pilot 1). Analyzes were performed according to the same procedure as described in subchapter 2.1.4.4.

3.2. Results

3.2.1 Flesh quality

Food is one of the basic needs of life which serves as the foundation for survival. The proper human diet should satisfy the requirements for energy and nutritive components including: essential polyunsaturated fatty acids, exogenous amino acids being the component of standard proteins, mineral components, fat, and water soluble vitamins. Prawns are valued by nutritionists because of the nutritional value and low caloric content. There are several species of shrimps available on the European market, which are breaded according to different practices and in different conditions. Recently, the development of innovative approaches to breeding aquatic organisms, for example based on RAS has been observed. Species as well as conditions of breading (e.g. quality of water, quality of feed) may impact the nutritional quality as well as safety of a product provided to consumers.

The objective of the project was to compare the nutritional quality of prawns available in the European market with the quality of prawns bred within the project in RAS system and to compare the nutritional value and levels of contaminants between different shrimp species. Levels of contaminants measured were referred to permissible limits established for food (Tab. 12).

Table 12. Permissible limits of contaminants established for food.

contaminant	Permissible limit	Source of reference
Hg	0,5 mg/kg	Commission Regulation 1881/2006
Pb	0,5 mg/kg	Commission Regulation 1881/2006
Cd	0,5 mg/kg	Commission Regulation 1881/2006
γ-HCH	200 ng/g	Council Directive 86/363/EEC
HCB	100 ng/g	Council Directive 86/363/EEC
DDTs	1000 ng/g	Council Directive 86/363/EEC
PCBs	75 ng/g	Commission Regulation 1259/2011/EEC

Chemical composition of aquatic species is determined by many factors. These are inherent factors related to the biology of the species, in particular with regard to eating habits and spawning and external factors related to environmental conditions and diet composition. Therefore, different species are characterized by a large variation in the chemical composition of the body. Significant differences can also be observed for one species, due to the habitat. In the case of organisms reared under controlled conditions, when proper water parameters are maintained, the main external factor determining the chemical composition is feed.

3.2.1.1. Comparison of contaminant levels between different shrimp species

As it is shown on figures below (Fig. 57 and 58), mean levels of some contaminants were higher in wild shrimps than in farmed ones. In case of DDTs the highest level was noted in indian kidi shrimp caught in in the western Indian Ocean (FAO 51) region, while in case of Cd the highest level was measured in red Argentinian shrimp caught in the south-west Atlantic (FAO 41). **Nonetheless the permissible limits of contaminants measured were not exceeded in any of samples examined.**

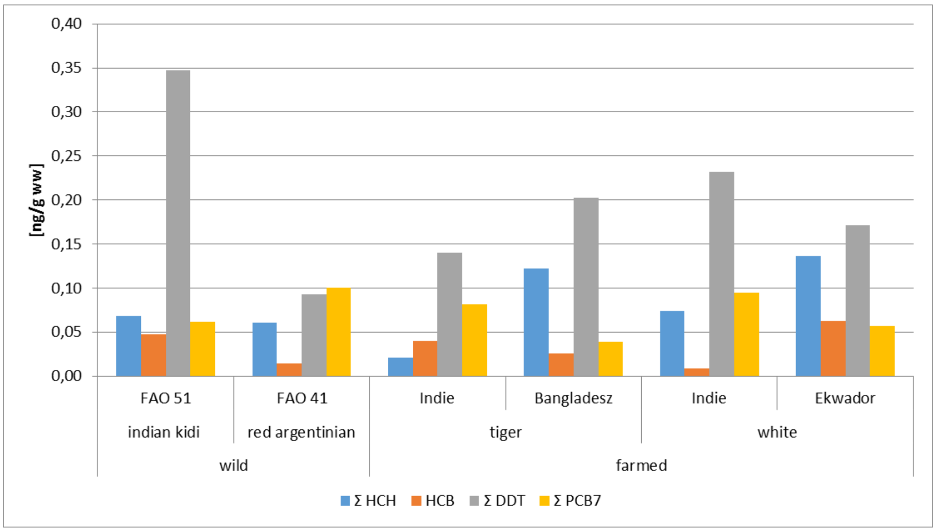


Fig. 57. Levels of pesticides and PCBs in shrimp samples collected from the Polish market.

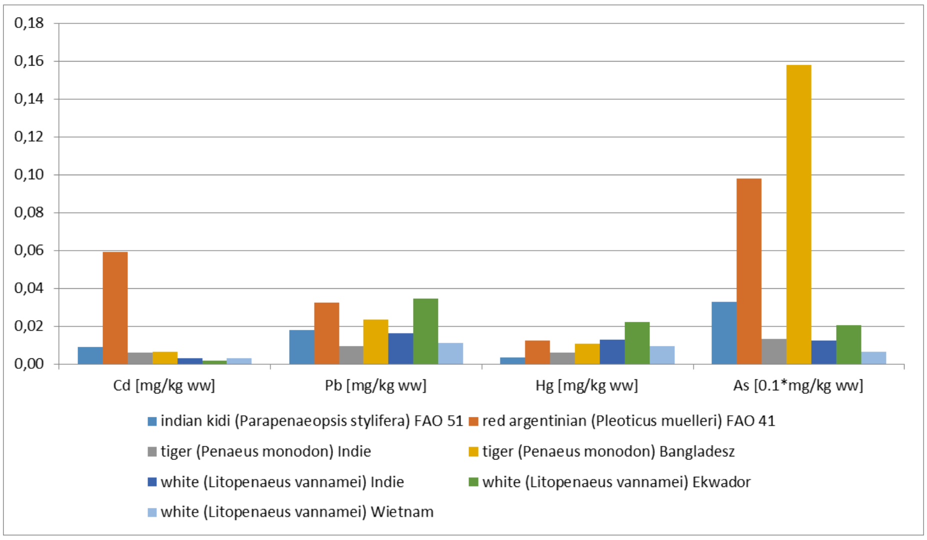


Fig. 58. Levels of metals in shrimp samples collected from the Polish market

3.2.1.2. Comparison of contaminant levels in white shrimp (*Litopenaeus vannamei*) collected from the Polish market and in shrimps bred in RAS system

<u>Metals</u>

Mean levels of cadmium were similar in *Litopenaeus vannamei* cultured in RAS (0.003-0.004 mg/kg) and in *Litopenaeus vannamei* imported from India (0.003 mg/kg), Vietnam (0.005

mg/kg) and Ecuador (0.002 mg/kg). Mean level of lead in *Litopenaeus vannamei* cultured in RAS was 0,01 mg/kg while in imported *Litopenaeus vannamei* were from 0.015 to 0.025 mg/kg. Mean levels of mercury were slightly higher in *Litopenaeus vannamei* cultured in RAS (0,03-0,05 mg/kg) than in *Litopenaeus vannamei* imported from India (0.012 mg/kg), Vietnam (0.01 mg/kg) and Ecuador (0.02 mg/kg).

<u>Organic contaminants</u>

Mean levels of PCBs *Litopenaeus vannamei* cultured in RAS were between 0.01 and 0.2 ng/g while imported *Litopenaeus vannamei* exhibited levels about 0.05 ng/g. Levels on organochlorine pesticides (shown in figures 59 and 60) were very similar in *Litopenaeus vannamei* cultured in RAS and imported from India, Vietnam and Ecuador.

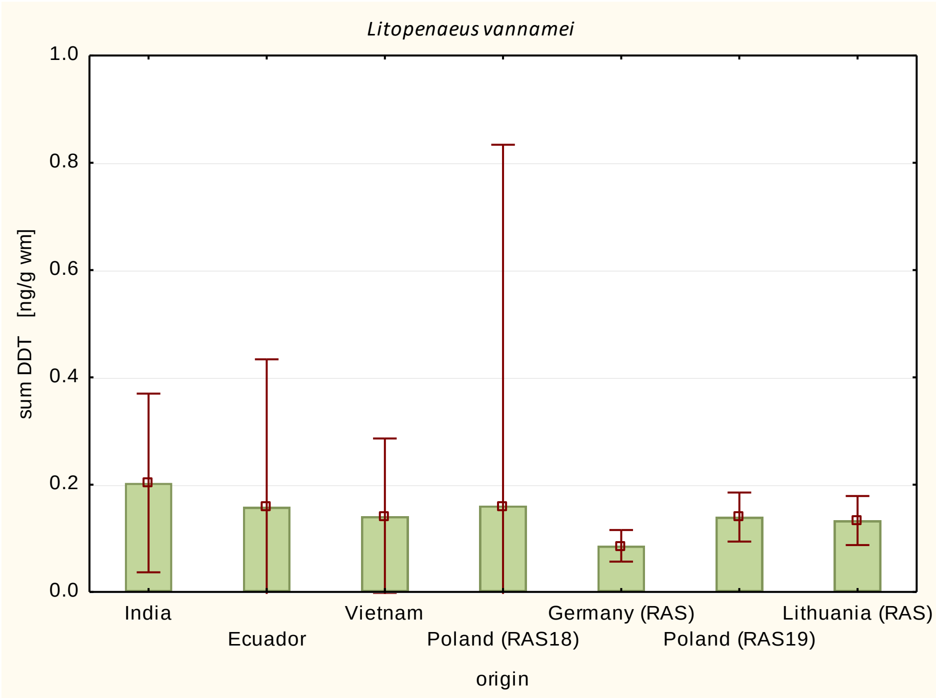


Fig. 59. Levels of DDTs in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

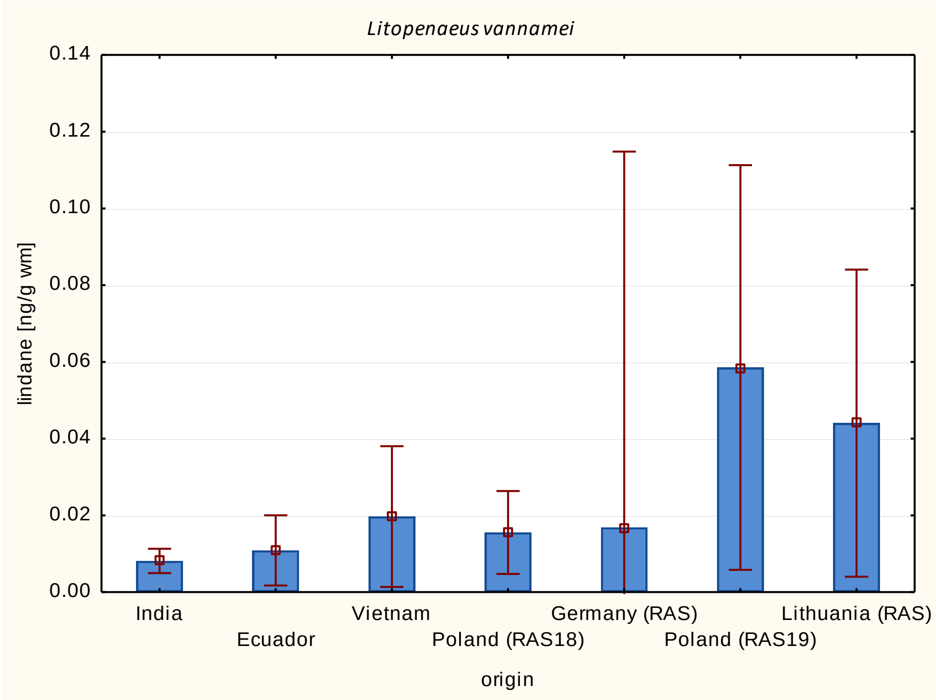


Fig. 60. Levels of lindane in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

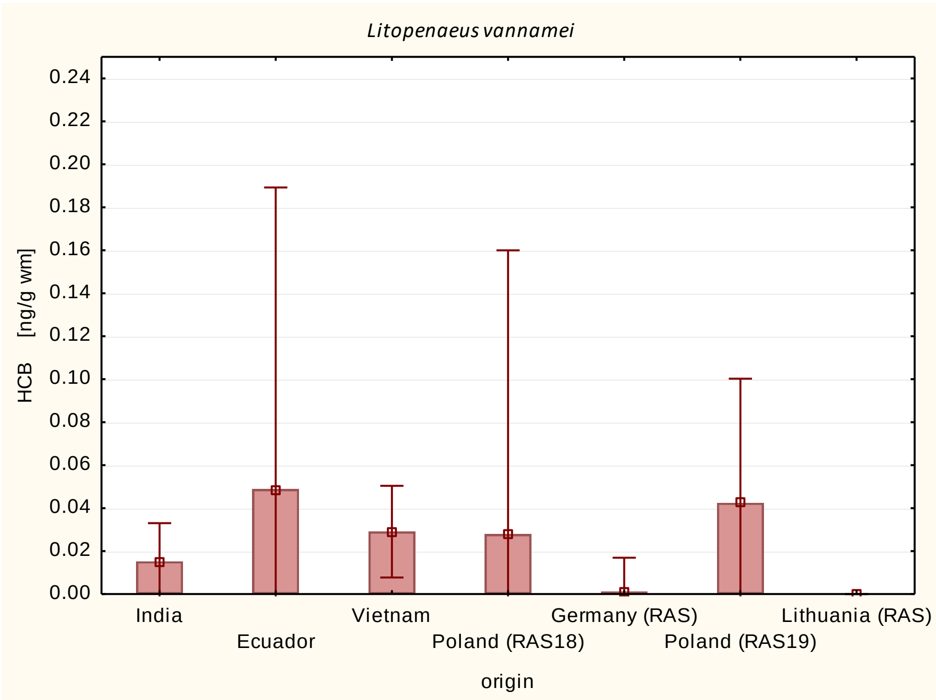


Fig. 61. Levels of HCB in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

Project results indicated that the levels of undesirable substances were very low in all species examined in relation to the applicable limits. Levels of hazardous substances in white shrimp (*Litopenaeus vannamei*) bred within the project were similar to those in white shrimp imported from India, Ecuador and Vietnam.

3.2.1.3 Comparison of quality of *Litopenaeus vannamei* sampled in the Polish market and farmed in RAS systems

The nutritional benefits of aquatic food stem for the most part, from its exceptionally advantageous fatty acids profile. In recent years, increasing attention has been focused on the significance of polyunsaturated fatty acids (PUFAs) in human nutrition, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These long-chain, polyunsaturated fatty acids (LC-PUFAs; acids comprising 20 or more carbon atoms and at least three double bonds) are the major components of cellular membranes. In addition EPA and DHA play an extremely important roles in regulating biochemical and physiological processes. Importantly, EPA and DHA are found in significant amounts only in foods of aquatic origin. The specific pro-health properties of aquatic food in the diet are mainly determined by the absolute content of eicosapentaenoic acid (20: 5n-3, EPA) and docosahexaenoic acid (22: 5n-3, DHA) and the ratio of the n-3 to n-6 fatty acids.

A diet containing the required amounts of EPA and DHA and the proper proportions between the amount of n-3 and n-6 acids ensures the proper homeostasis of the body and protection against certain diseases that have become common in today's society, such as cardiovascular, neurodegenerative, cancerous diseases and obesity , depression or type 2 diabetes (Fig. xxx). **The daily requirement for EPA and DHA for prevention of heart diseases is 500 mg.**

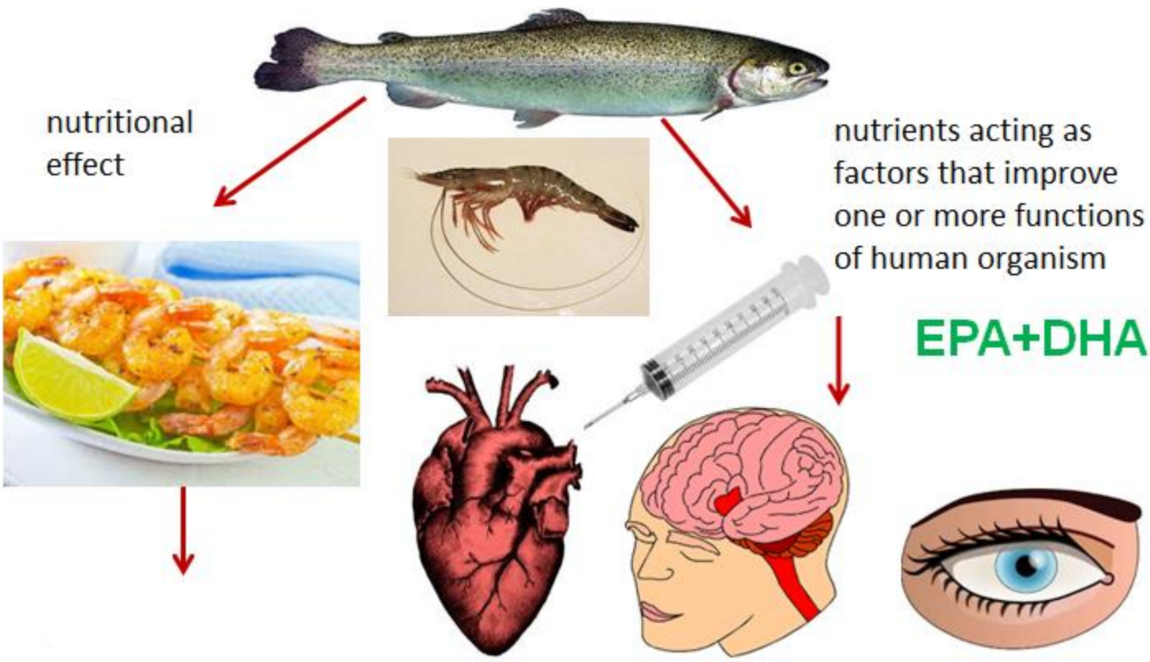


Fig. 62 Effects of EPA and DHA on the body homeostasis

As it is presented in the figure below (Fig. 63) fat content was very similar in *Litopenaeus vannamei* farmed in RAS and in *Litopenaeus vannamei* imported from Asia. **What is interesting, fat content in shrimps from experimental culture conducted in UG and shrimps obtained from commercial RAS farm from Germany were practically the same, despite the fact that shrimp from commercial farm were fed with formula in which fat content was much lower (4%) than in feed used in the experiment (11%).**

Similar observation was made for vitamins: higher level in feed didn't result in higher levels in shrimps(results shown in figures). Levels of EPA and DHA (Fig. 64) were two-fold higher in shrimps bred in RAS than in shrimps imported from Asia. Also content of vitamin A was higher in shrimps from RAS than in imported shrimps sampled in the Polish market (Fig. 65). In turn levels of vitamin D3 were higher in *Litopenaeus vannamei* imported from Asia (Fig. 67).

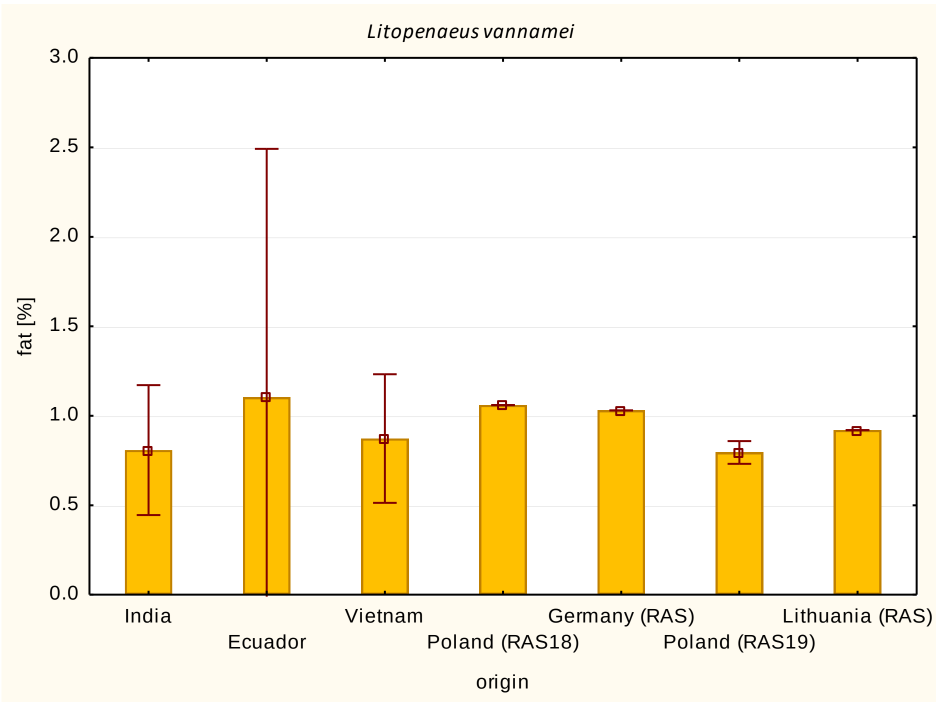


Fig. 63. Fat content in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

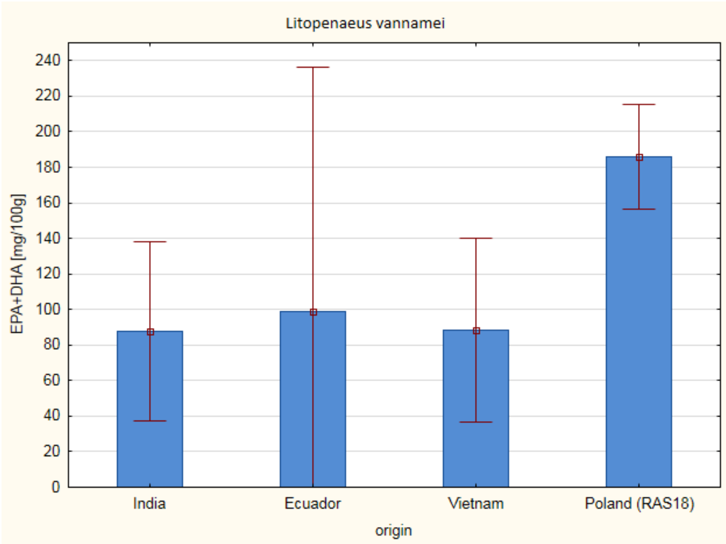


Fig. 64. Fatty acids concentration in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

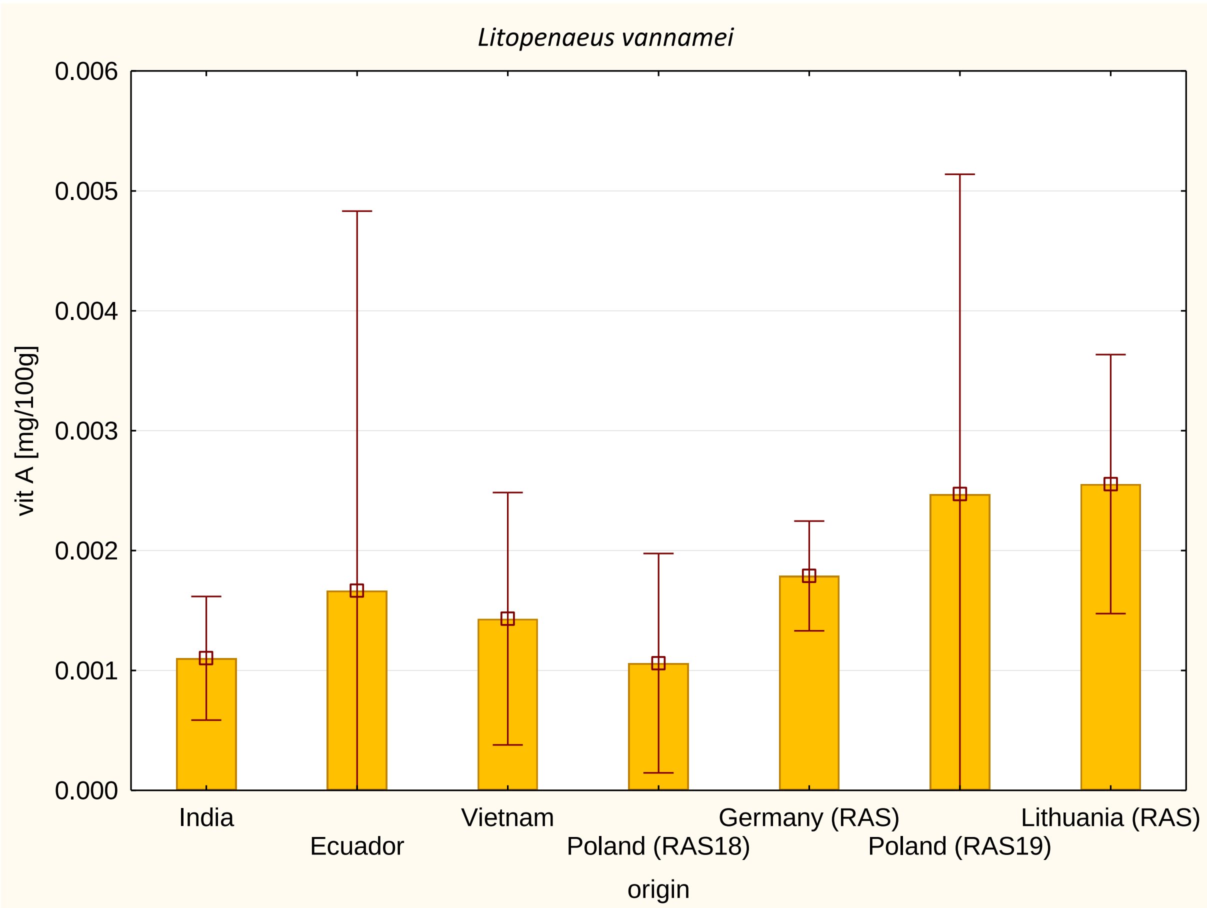


Fig. 65. Levels of vitamin A in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

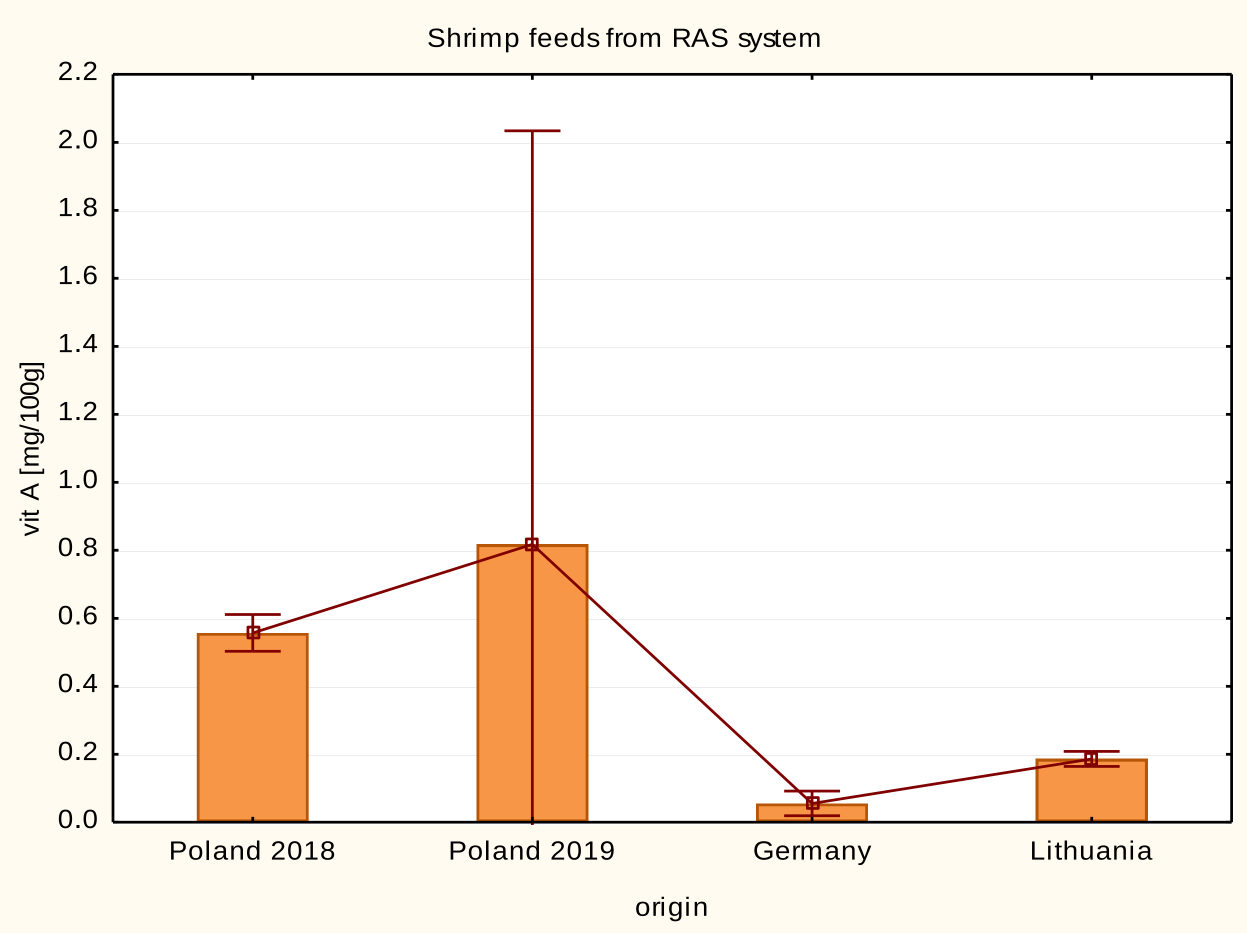


Fig. 66. Levels of vitamin A in feed for *Litopenaeus vannamei* farmed in RAS system.

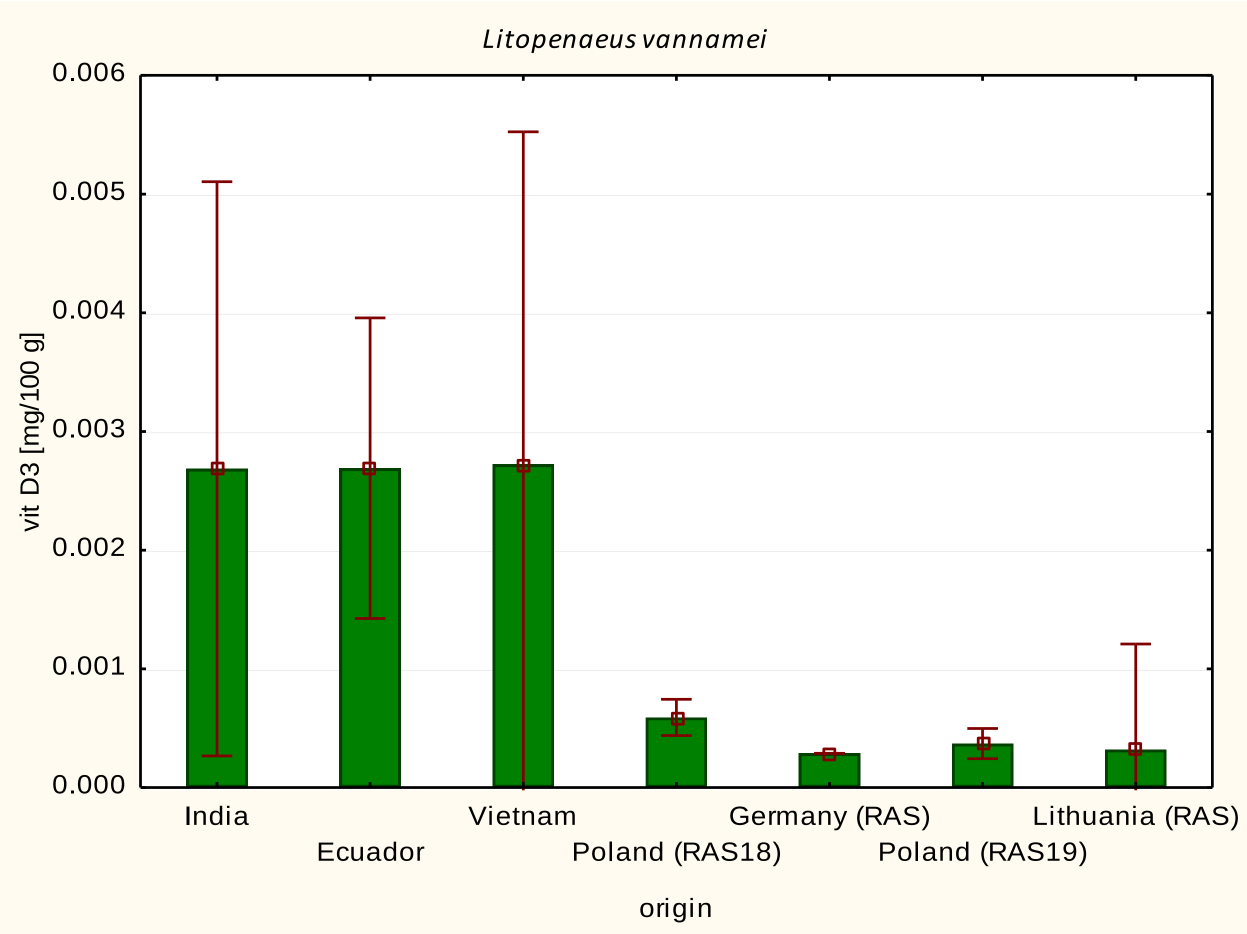


Fig. 67. Levels of vitamin D_3 in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

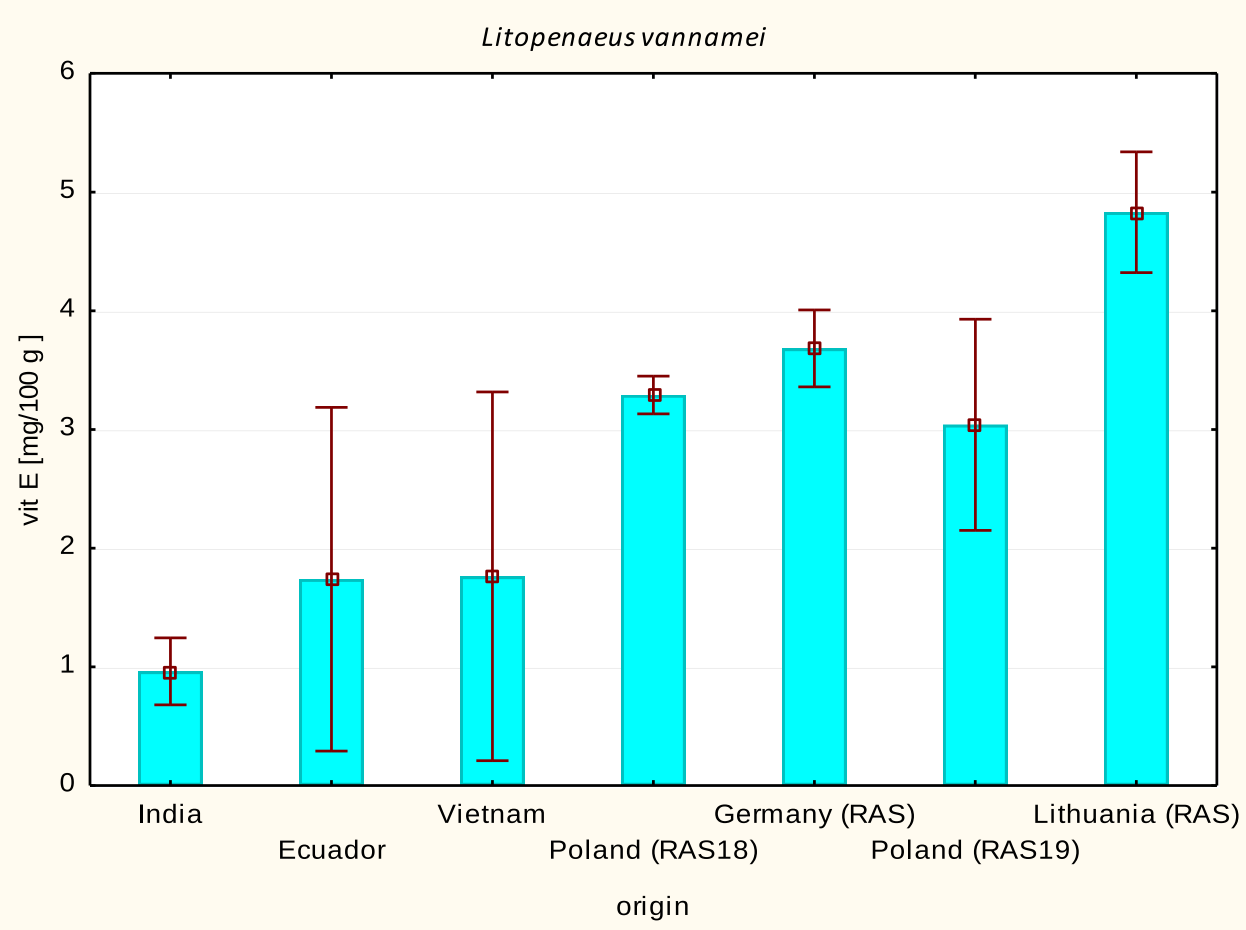


Fig. 68. Levels of vitamin E in *Litopenaeus vannamei* collected from the Polish market and in shrimps bred in RAS system.

Project results indicated that shrimps bred as part of the project were characterized by a similar level of nutrients as the shrimp of the same species originated from Asia, although it is worth paying attention to the higher level of EPA and DHA, allowing the placement of a nutritional health claim on a product.

3.2.2. Organoleptic properties

3.2.2.1 Shrimps from Polish market

Batch INA-18

The analysed batch of shrimps INA -18 was delivered frozen with the glaze (25%) as a headed shrimp (without carapace) as „easy pell" shrimp. The producer used the stabilizers E451 (triphosphates) and E542 (probably it should be E452- polyphosphates) and salt (NaCl) to minimize the quality changes during storage. Triphosphates and polyposphates also limit the leakage of water after thawing, thereby increasing the weight of the product. The results of the morphometric are presented in Table 13, whereas of sensory tests on Fig. 69-73.

Table 13. Average mass and length of analyzed shrimps from batch INA-18.

Parameter	Length [cm]	Mass [g]
Average ±SD	9.20 ± 0.39	15.88 ± 1.23
X_{min}	9.00	13.51
X_{max}	10.00	18.50
The total number of the shrimps in analised batch [pcs]	23	
The number of the shrimps in kg [psc/kg]	62.97	

Fig. 69. The analysed batch INA-18 after defrosting (Photo credit: Olga Szulecka).

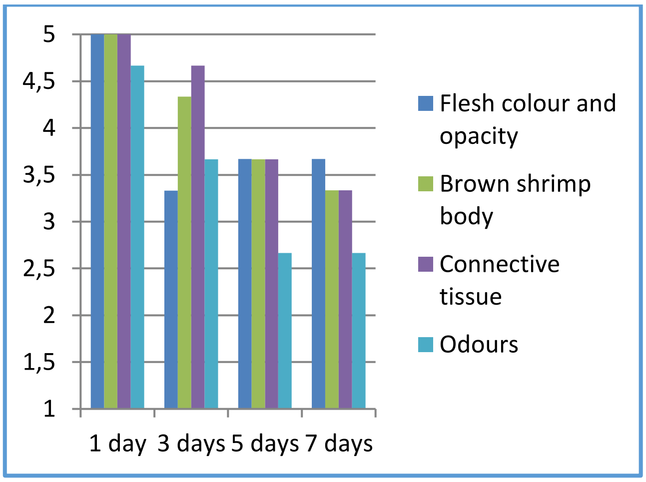


Fig. 70. Sensory assessment of frozen (raw) shrimps (INA-18) after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 71. Defrosted (raw) shrimps from batch INA-18 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

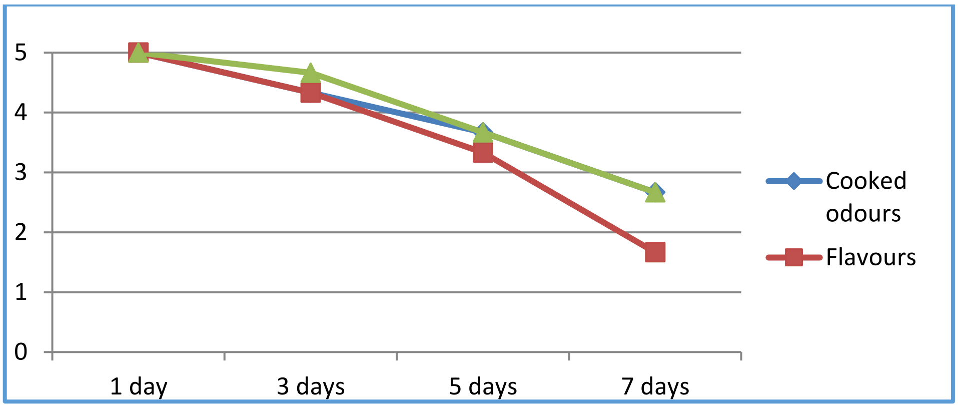


Fig. 72. Sensory assessment of frozen (cooked) shrimps (INA-18) after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 73. Defrosted (cooked) shrimps from batch INA-18 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The results of the analyses of frozen shrimp *L. vannamei* for batch INA-18 shown that the addition 25% of glaze and addition of triphosphates and polyphosphates to the product allow reducing the adverse changes of shrimp quality. The shrimp appearance, taste, odour and texture after 7 days of storage was very acceptable. The shrimps after a week of storage were still highly rated and can be used for consumption. Fast removing of carapace improves the quality of shrimp during the frozen storage.

Batch INA-19

The batch INA-19 was also delivered frozen with 20% of glaze, without carapaces. The producer wrote on the label that used salt (NaCl) and stabilizers: E451 (triphosphates) and E452 (polyphosphates). Those substances minimize the quality changes and maintain a higher mass of the product after defrosting. The results of the morphometric are presented in Table 14, whereas of sensory tests on Fig. 74-79.

Table 14. Average mass and length of analyzed shrimps from batch INA-19

Parameter	Lenght [cm]	Mass [g]
Average ±SD	8.17 ± 0.54	9.10 ± 1.12
X_{min}	7.00	7.25
X_{max}	9.00	10.97
The total number of the shrimps in analised batch [pcs]	23	
The number of the shrimps in kg [psc/kg]	109.87	

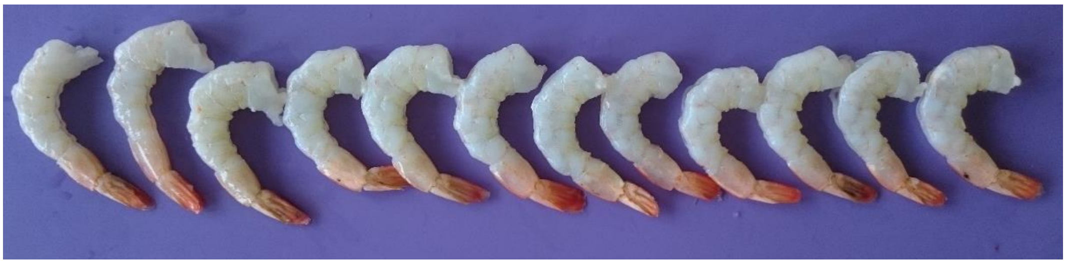


Fig. 75. The analysed batch INA-19 after defrosting (Photo credit: Olga Szulecka).

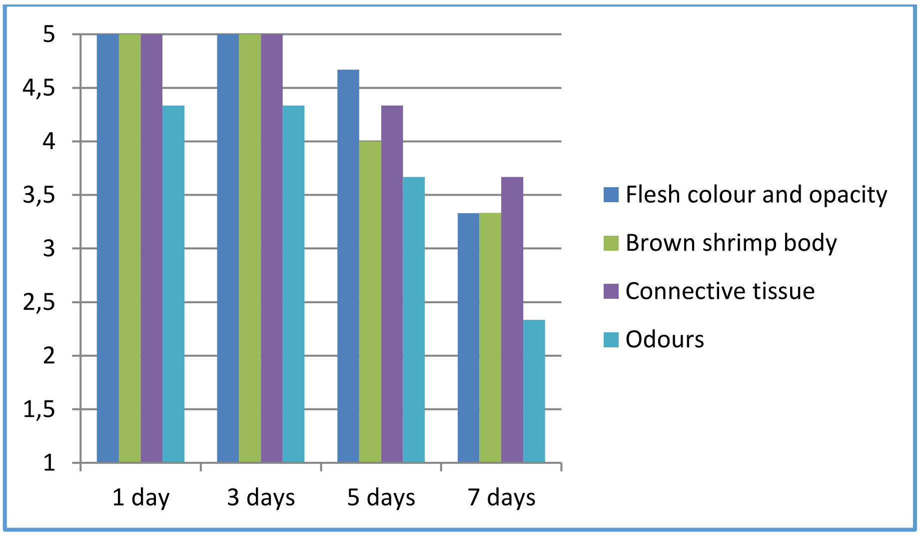


Fig. 76. Sensory assessment of frozen (raw) shrimps (INA-19) after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 77. Defrosted (raw) shrimps from batch INA-19 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

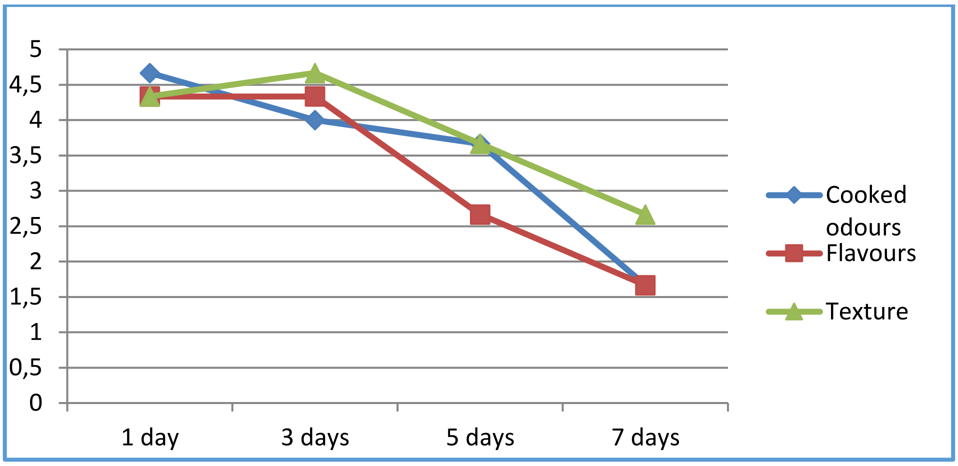


Fig. 78. Sensory assessment of frozen (cooked) shrimps (INA-19) after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 79. Defrosted (cooked) shrimps from batch INA-19 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The results of the analysed of INA-19 batch show that using stabilisers E451 and E452 allow maintaining the good quality of shrimp. The analysed batch after 7 days of storage was in very good condition and could be eaten by consumers.

Batch INA-22

The batch INA-22 from the market was delivered as frozen, headed shrimps with 30% of glaze. The producer added salt (NaCl) and stabilizer E451 (triphosphates) to minimize the quality changes during storage and loss of weight after defrosting. The results of the morphometric are presented in Table 15, whereas of sensory tests on Fig. 80-84.

Fig. 80. The analysed batch INA-22 after defrosting (Photo credit: Olga Szulecka).

Table 15. Average mass and length of analyzed shrimps from batch INA-22.

Parameter	Lenght [cm]	Mass [g]
Average ± SD	1130 ± 2.68	22.04 ± 2.09
X_{min}	9.00	18.31
X_{max}	20.50	25.87
The total number of the shrimps in analised batch [pcs]	15	
The number of the shrimps in kg [psc/kg]	45.37	

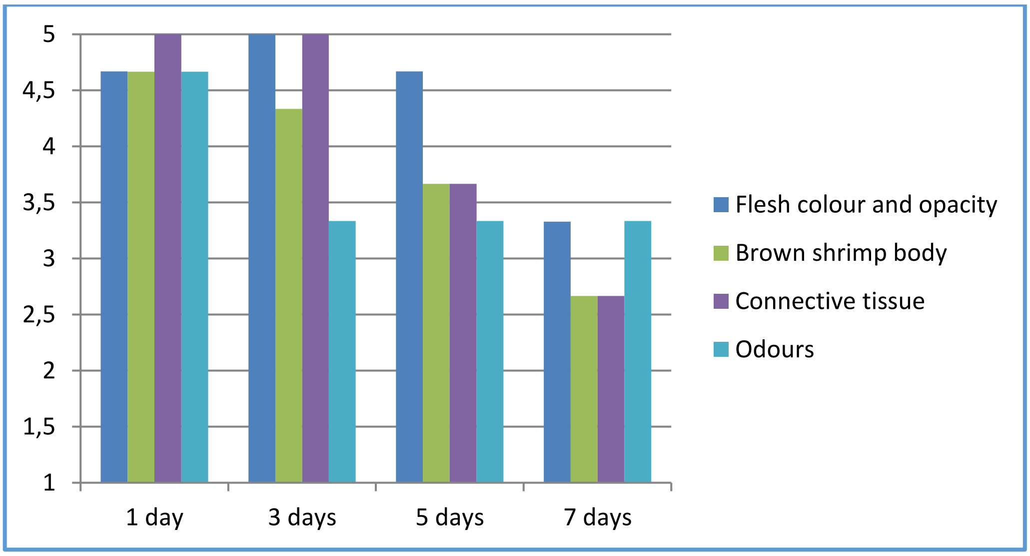


Fig. 81. Sensory assessment of frozen (raw) shrimps (INA-22) after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 82. Defrosted (raw) shrimps from batch INA-22 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

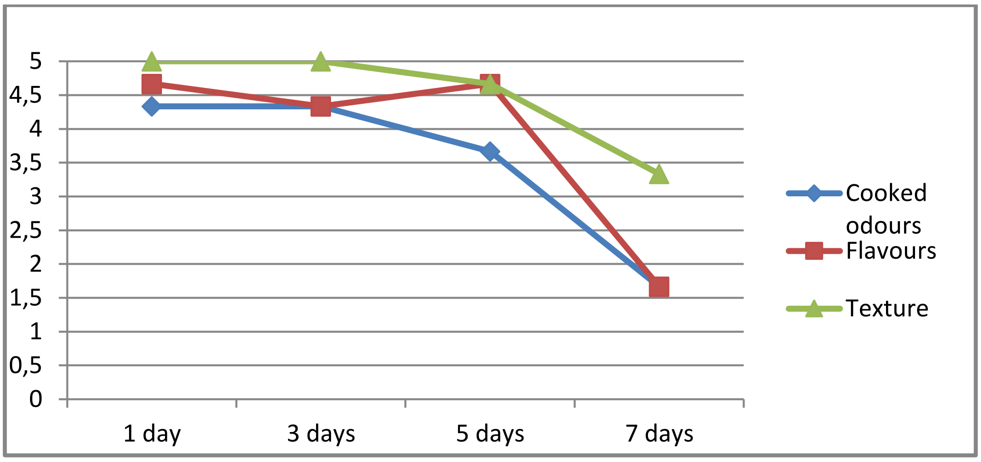


Fig. 83. Sensory assessment of frozen (cooked) shrimps (INA-22) after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 84. Defrosted (cooked) shrimps from batch INA-22 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The quality of the analysed shrimps *L. vannamei* from batch INA-22 was very acceptable during the whole storage period. The texture of cooked shrimp after a week of storage was ranked for more than 3 points, only flavours and odours at the end of research was noted lower.

Batch INA-23

Batch INA-23 was delivered fresh, iced. The batch for testing was headed to analyse the headed fresh shrimps quality changes during storage. The average yield of headed shrimps was 68.75 ± 4.51%. The results of the morphometric are presented in Table xxx, whereas of sensory tests on Fig. 85-88.

Table 16. Average mass and length of analyzed shrimps from batch INA-23 .

Parameter	Lenght [cm]	Mass [g]	Mass after heading [g]
Average ± SD	14.06 ± 0,53	19.55 ± 1,83	13.44 ± 1.58
X_{min}	12.50	15.58	10.90
X_{max}	15.00	23.03	19.08
The total number of the shrimps in analised batch [pcs]	30		30
The number of the shrimps in kg [psc/kg]	51.15		74.39

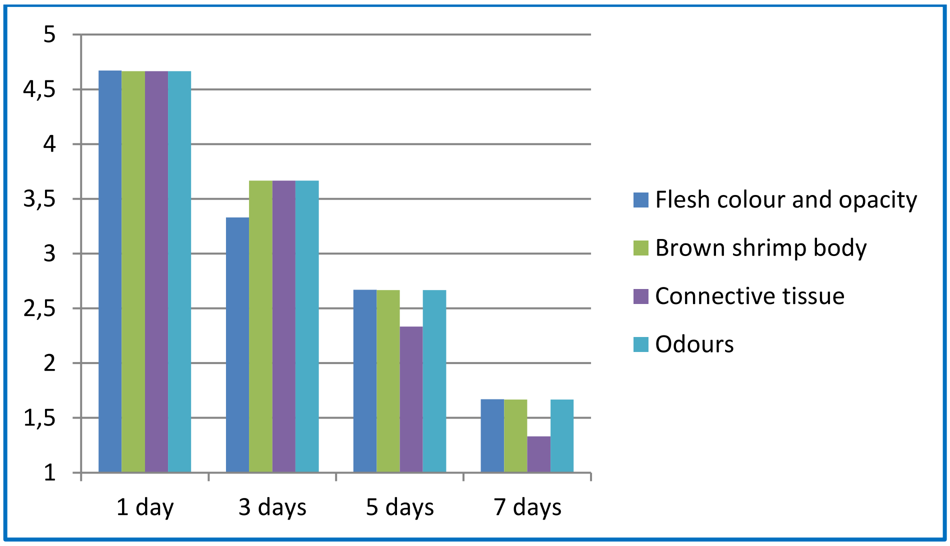


Fig. 85. Sensory assessment of fresh (raw) shrimps (INA-23) after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 86. Fresh (raw) shrimps from batch INA-23 after 1 and 7 days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

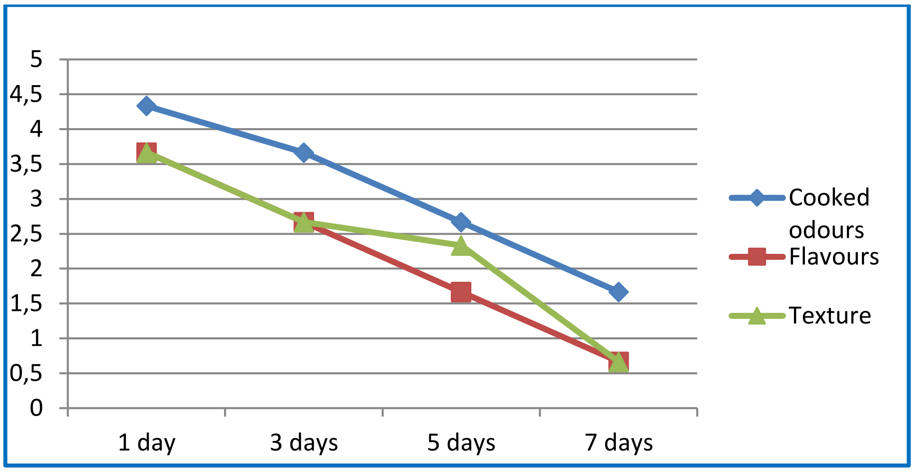


Fig. 87. Sensory assessment of fresh (cooked) shrimps (INA-23) after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 88. Fresh (cooked) shrimps from batch INA-23 after 1 and 7 days of storage in temperature of 1÷3°C.

The analysed shrimps were sold as fresh (but they could be probably defrosted before selling) and the quality of them was much lower after a week of storage than the frozen shrimps with stabilizers. The flavour and texture of shrimps on the 7th day of storage were ranked lower than 1 point.

Batch INA-24

The batch of shrimps *Litopenaeus vannamei*, batch INA-24 was also delivered fresh, iced. The shrimps weren't headed to test the quality of the headed shrimp during storage. The results of the morphometric are presented in Table 17, whereas of sensory tests on Fig. 89-93.

Table 17. Average mass and length of analyzed shrimps from batch INA-24.

Parameter	Lenght [cm]	Mass [g]
Average ± SD	14.89 ± 0.77	23.93 ± 3.93
X_{min}	13.50	15.45
X_{max}	17.00	30.98
The total number of the shrimps in analised batch [pcs]	33	

The number of the shrimps in kg [psc/kg]	41.78

Fig. 89. The analysed batch INA-24.

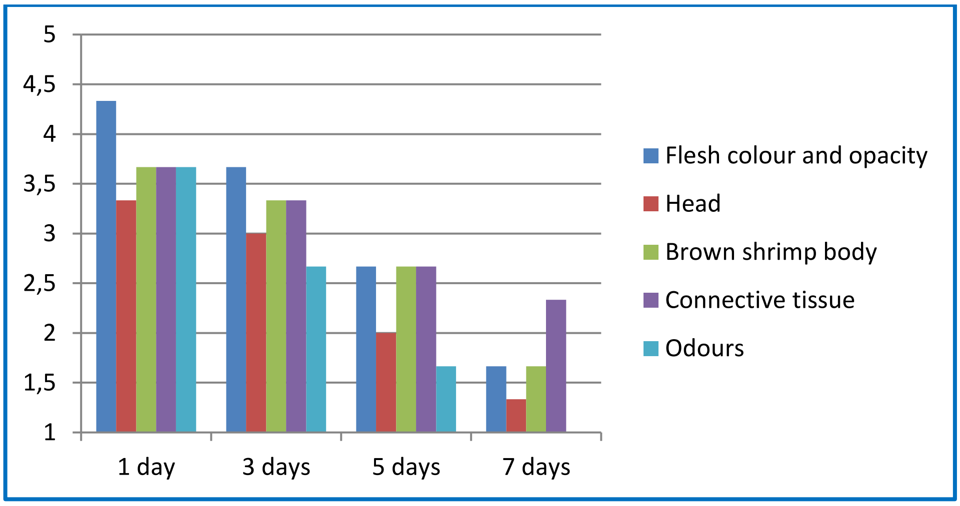


Fig. 90. Sensory assessment of fresh (raw) shrimps (INA-24) after 1,3,5 and 7 days of storage in temperature of 1-3°C.

Fig. 91. Fresh (cooked) shrimps from batch INA-24 after 1 and 7 days of storage in temperature of 1÷3°C.

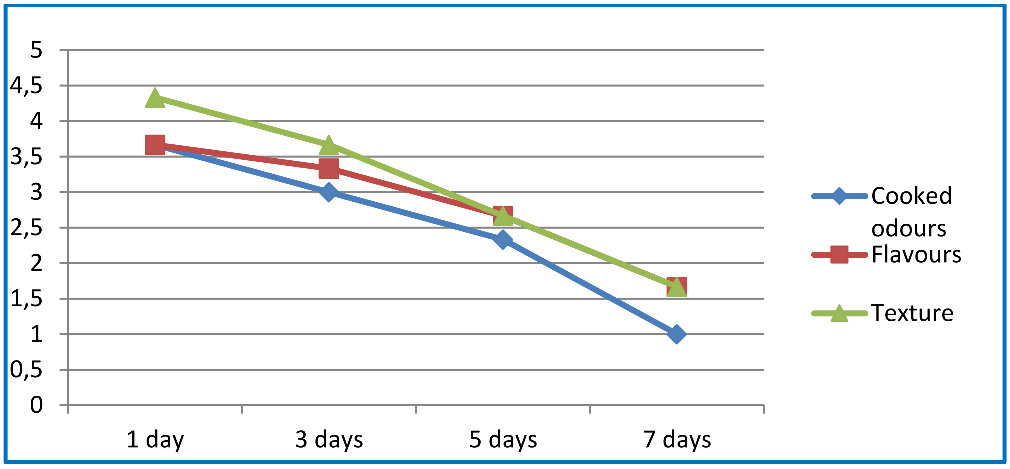


Fig. 92. Sensory assessment of fresh (cooked) shrimps (INA-24) after 1, 3, 5, 7 days of storage in temperature of 1÷3°C.

Fig. 93. Fresh (cooked) shrimps from batch INA-24 after 1 and 7 days of storage in temperature of 1÷3°C.

The initial quality of the shrimps was lower than the batch INA-23. The quality of the whole shrimps was lowering much more quickly than headed ones.

3.2.2.2 Shrimps from RAS in Lithuania (Pilot 1)

The batch of shrimps from RAS in Lithuania (Pilot 1) was delivered whole and fresh, iced (Fig. 94). The shrimps were in very good condition. During the survey, the quality changes of whole and headed shrimps were tested. The half of analyzed batch of shrimps was headed and the average mass of the headed part of batch amounts 15.31 ± 2.08 g and the average yield of it was 65.24 ± 1.98%.

Fig. 94. The analyzed batch of shrimps from Lithuania (Photo credit: Olga Szulecka).

The results of the morphometric analyzes of shrimps from Lithuania are presented in the Table 18, whereas of sensory tests on the figures 95-98.

Table 18. Average mass and length of analyzed shrimps from RAS from Lithuania.

Parameter	Lenght [cm]	Mass [g]
Average ±SD	15.53±0.78	25.81±3.02
X_{min}	14.00	21.50
X_{max}	17.00	30.69
The total number of the shrimps in analised batch [pcs]	16	
The number of the shrimps in kg [psc/kg]	38.75	

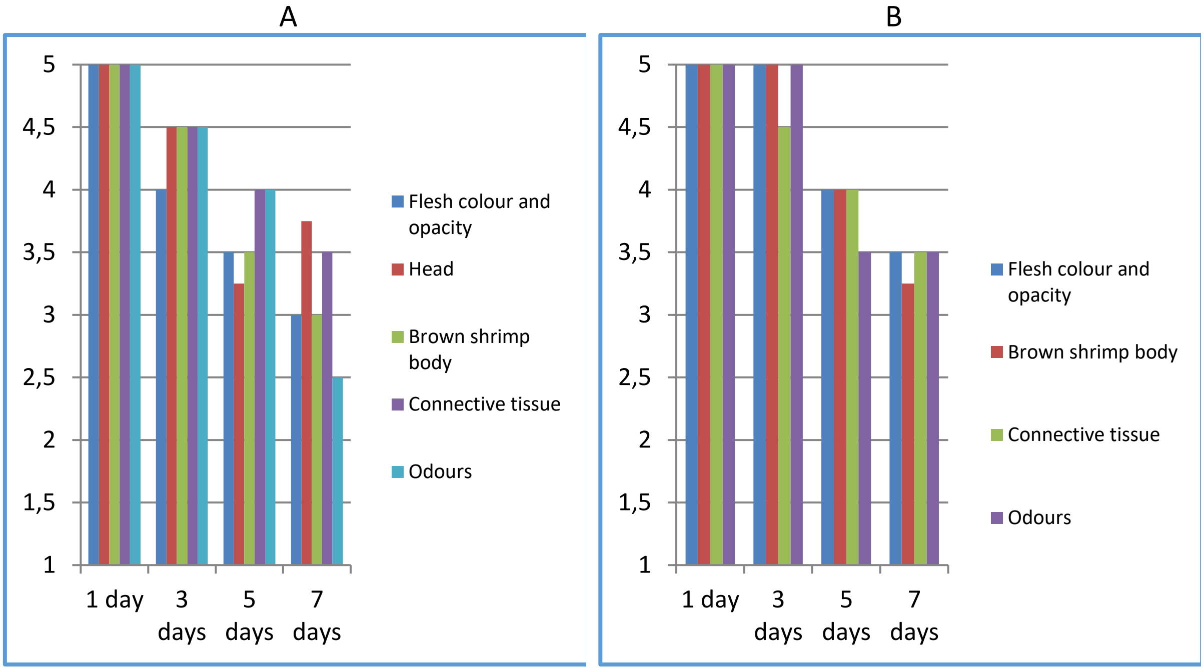


Fig. 95. Sensory assessment of fresh (raw) of whole (A) and headed (B) shrimps from RAS in Lithuania after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 96. Fresh (raw) shrimps from batch from RAS in Lithuania after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

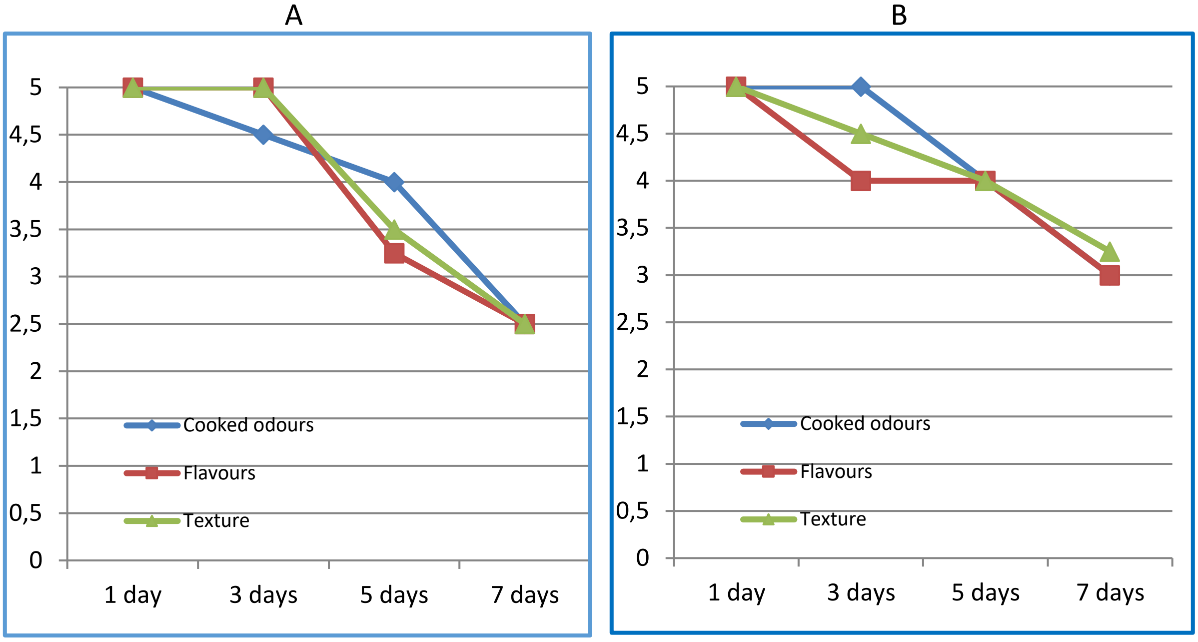


Fig. 97. Sensory assessment of fresh (cooked) whole (A) and headed (B) shrimps from RAS in Lithuania after 1,3,5 and 7 days of storage in temperature of 1÷3°C.

Fig. 98. Fresh (cooked) shrimps from batch from RAS in Lithuania after 1 (A) and 7 (B) days of storage in temperature of 1÷3°C (Photo credit: Olga Szulecka).

The shrimps from Lithuania were in very good condition when were delivered and also after 7 days of storage and can be eaten by consumers for the whole analysed time of storage. The higher ranks were obtained by the headed shrimps (both raw and cooked), which allows saying that heading of shrimps is very important to maintain the quality during storage.

4. Potential of crustacean RAS production in Pomerania

4. 1 Preliminary analysis of socio-economic issues

4.1.1 Restaurants perception

Shrimp surveys in Tri-City restaurants

The surveys were carried out in March 2019, through interviews based on questionnaires prepared by InnoAquaTech project team from the Institute of Oceanography, University of Gdańsk, Poland.

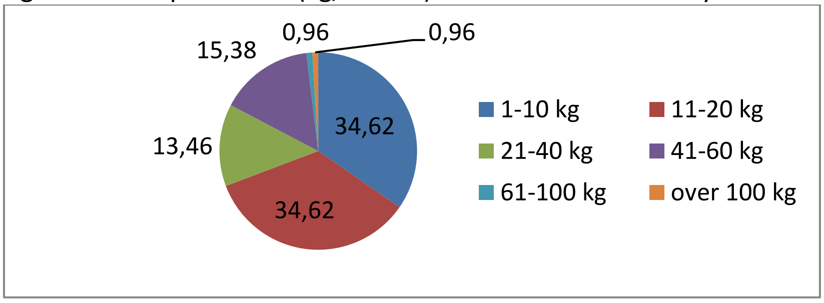
The survey was carried out among 104 representatives of restaurants from Tri-City, Poland (Gdańsk, Sopot, Gdynia).

The questionnaire consisted of 19 questions on the product (shrimps) and consumers, and 3 questions on the restaurants (location, type of cuisine, type of clients) and 1 question on the position of the person providing the answers.

Conclusions on shrimps in the restaurants' offer:

Demand for shrimps in Tri-City restaurants: The analysis of the questions on shrimp demand shows that it is low. 35% of the surveyed restaurants use 1 to 10 kilograms of shrimps per month. Another 35% use from 11 to 20 kilograms of shrimps per month, which indicates that 70% of the surveyed restaurants use only up to 20 kilograms of shrimps per month. Only 28% of restaurants use between 21 and 60 kilograms per month. Only one of the surveyed restaurants declared using between 61 and 100 kilograms per month (sushi restaurant) and one – over 100 kilograms per month (Mediterranean restaurant).

Fig. 99 - Shrimp demand (kg/month)in the examined Tri-city restaurants



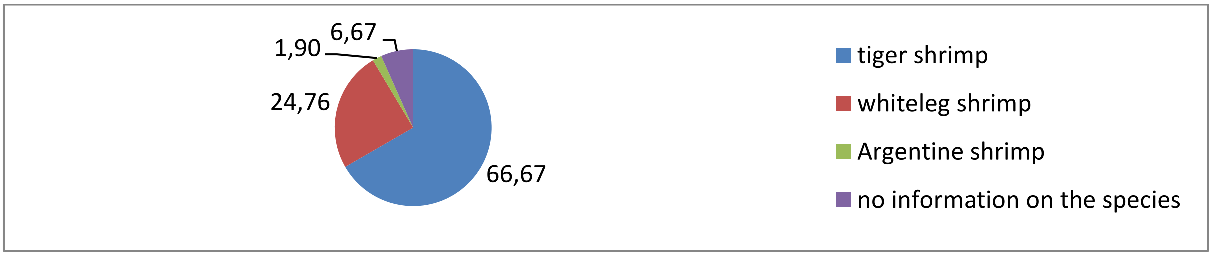
Shrimp species: Tiger shrimps turned out to be most frequently sold species in restaurants.

Since in the question regarding shrimp names, a surveyed person could provide either a market name of the shrimp or the species name, an assumption was made before further interpretation, therefore provided answers (whiteleg shrimp, royal shrimp, cocktail shrimp, gray shrimp, Argentinean shrimp) were classified as follows:

*tiger shrimp – species name, *Penaeus monodon*
whiteleg shrimp – species name, *Litopenaeus vannamei*
royal shrimp – market name for tiger shrimps (*Penaeus monodon*) and whiteleg shrimp (*Litopenaeus vannamei*), information from wholesale sites
cocktail shrimp – small shrimps sold as peeled and cooked, most often whiteleg shrimp (*Litopenaeus vannamei*), information from wholesale sites
gray shrimp – uncooked, different species, most possibly tiger shrimp (*Penaeus monodon*), information from wholesale sites
Argentine shrimp – species name, *Pleoticus muelleri*

After the above showed classification results for species only were obtained. The data indicates that tiger shrimps are defiantly most popular in the surveyed restaurants: 67% of the restaurants indicated the offer of this species, 25% - whiteleg shrimp, 2% - Argentinean shrimp, and the rest of restaurants cannot provide either the species or market name of shrimps sold.

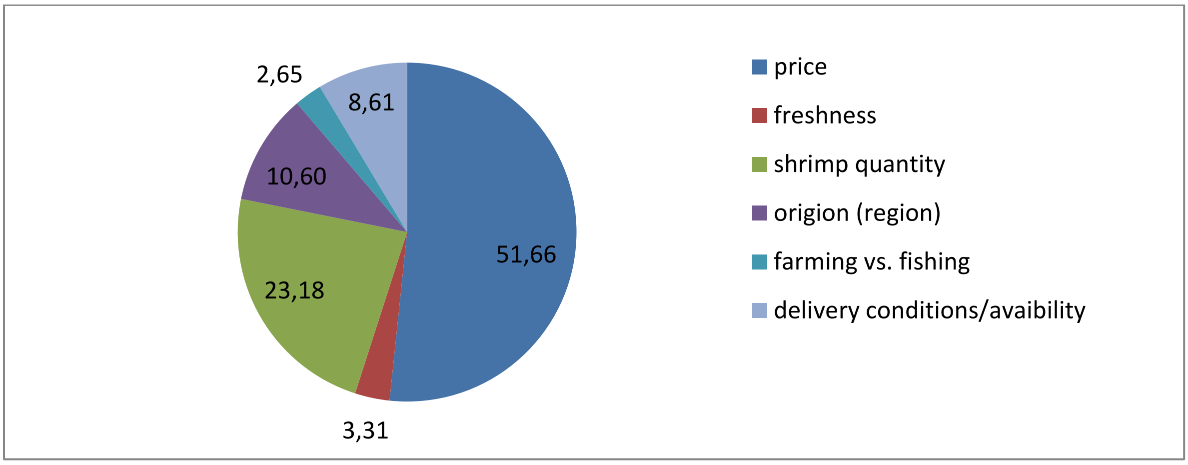
Fig. 100 - Shrimp species shares in sales in examined restaurants *



Shrimps delivery: Majority of the shrimp supplies come from suppliers, wholesalers. When asked about the method of shrimps delivery, the restaurateurs responded as follows: 76% of the restaurateurs buy shrimps from suppliers - wholesalers (of which 72% are general wholesalers, and 28% are fish wholesalers), and only 24% make direct purchases (of which 92% in supermarkets, and only 8% in market halls).

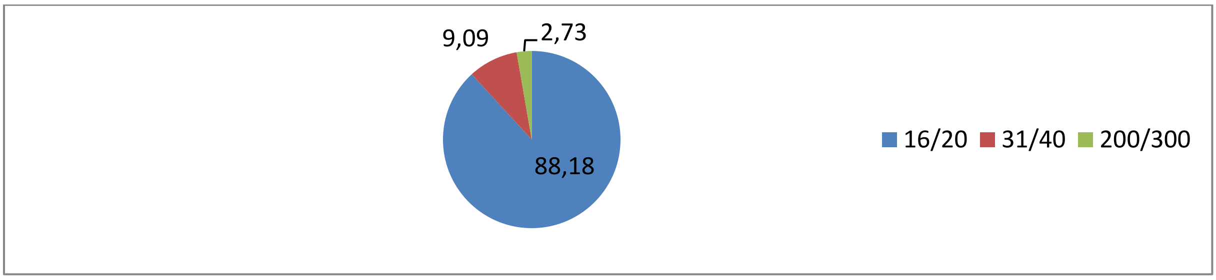
Factors influencing the decision of shrimps purchase: The most influential factor in decision making when buying shrimps is price. Restaurateurs were asked what factors are of the greatest importance when ordering shrimps to restaurants. The collected data show that the price of the product is a factor which is highlighted by the largest number of respondents – 52%. Second most important factor was shrimp quality –23% of all responses, followed by the shrimps origin (region) –11%, and delivery conditions/availability – 9%, freshness and farming vs. fishing constitute for only 3% of responses each. Interestingly, an information about sustainable catchment was never listed as a decisive factor.

Fig. 101 – Decisive factors in shrimps purchasing by restaurateurs



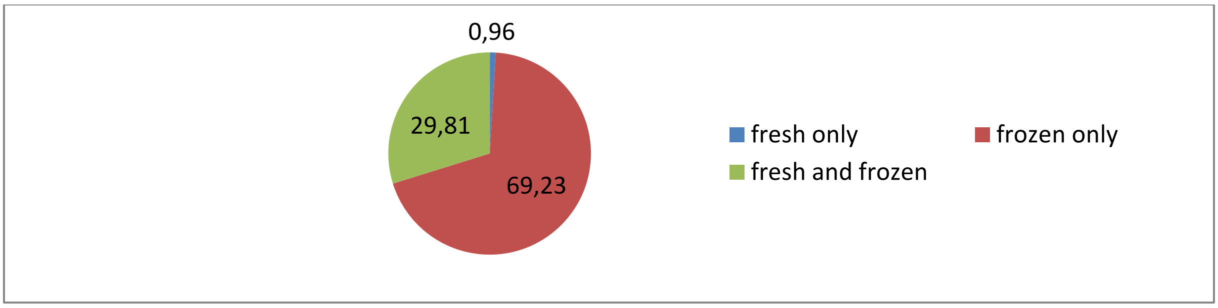
Shrimps' size: Restaurateurs prefer large shrimps. By far the most preferred shrimp size is 16/20, as many as 88% of the answers indicate the choice.

Fig. 102 - Preferred size of shrimps purchased by restaurateurs



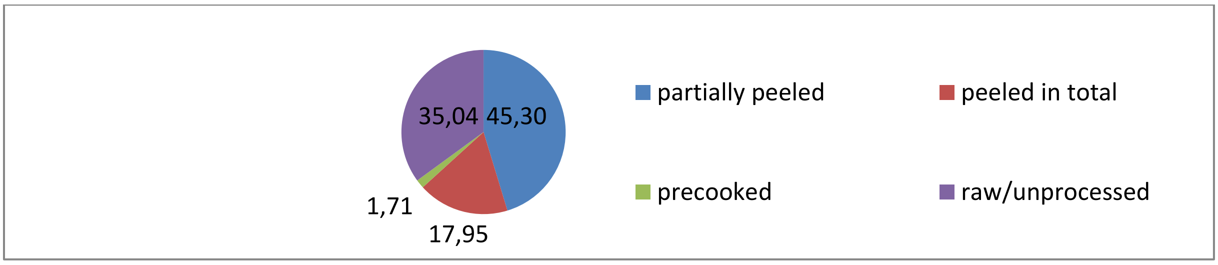
Fresh or frozen: Frozen shrimps are by far most popular. When asked about the type of shrimps bought (fresh/frozen), the following answers were obtained: 69% of respondents buy frozen shrimps only, 30% fresh and frozen, and only 1% fresh shrimps only.

Fig. 103 - Participation of restaurateurs in the purchase of fresh and frozen shrimps



Raw or processed: majority of the restaurants serve in their offer shrimps already processed before purchase. 65% of restaurateurs buy shrimps that have already been processed. In case of 45% restaurateurs, these shrimps are partially peeled, in case of 17% - peeled in total, in case of 2% - precooked. 35% of restaurateurs buy raw, unprocessed shrimps.

Fig. 104 – Participation of restaurateurs in the purchase of raw and processed shrimps



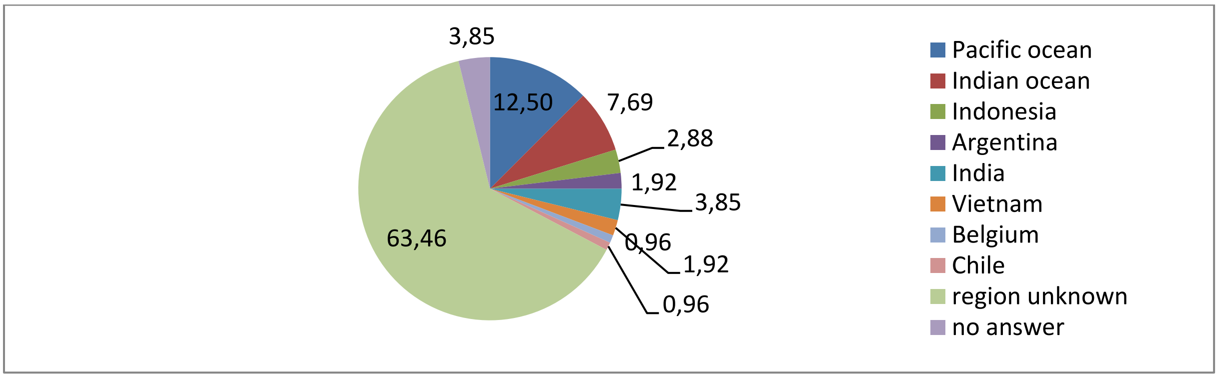
Shrimps in the menu: On average over five shrimps courses are offered by a single restaurant. When it comes to the sale of shrimp dishes in the restaurants surveyed, there are on average 5.5 shrimp dishes offered. The average price of a shrimp entry dish in the restaurants studied is 29 PLN, and the average price of a shrimp main course is 46 PLN. 87.5% of restaurateurs do not promote shrimps. 12.5% of restaurateurs promote shrimps through price, as dishes or chef's recommendation. 40% of restaurateurs answer that the sale of shrimps is also associated with seasonality, but actually it is the number of guests (higher number of guests during summer months), which is a factor. 60% responds that there is no dependence. As many as 83% of restaurateurs admit that customers do not ask questions about ordered shrimps. 17%

of restaurateurs answer that customers ask questions about the shrimp they consume, 50% of which are questions about their freshness. The rest is about size, quantity, origin and species.

Conclusions on restaurateurs' awareness on shrimps:

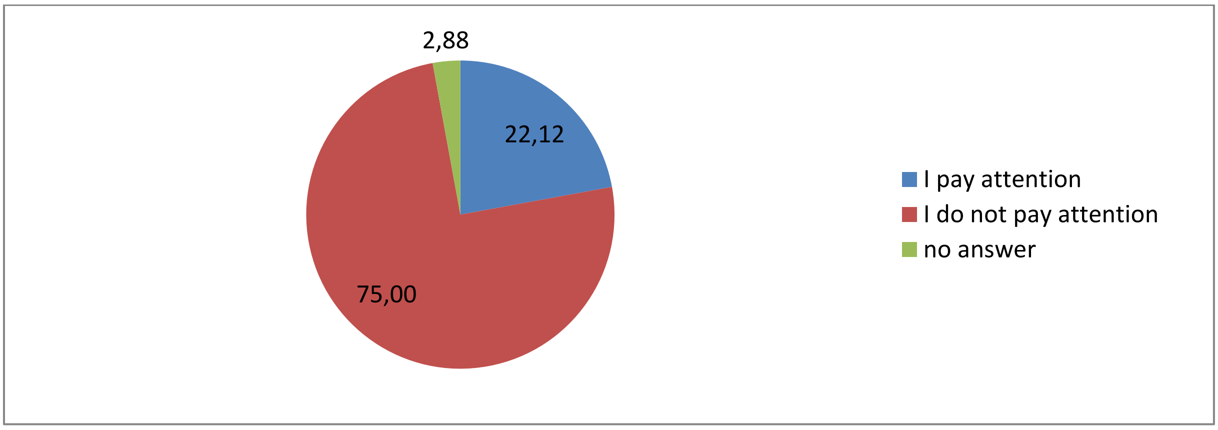
Origin of shrimps (region): Over half of the responding restaurateurs is not aware of the region of shrimps origin. Surveys show that the majority (63% of answering restaurateurs), do not know what region/country the shrimps come from. 12% of the restaurants' representatives say the shrimps they sell come from the Pacific region, and 8% from the Indian Ocean region. The remaining respondents indicate: India (4%), Indonesia (3%), Argentina (2%), Vietnam (2%), Chile (1%), and Belgium (1%). 4% of respondents did not respond.

Fig. 105 – Restaurateurs' awareness on shrimps origin (region/country)



Farming vs. fishing: For the majority of the restaurateurs the method of shrimp harvesting (fishing or farming) is irrelevant. As many as 75% of restaurateurs do not pay attention whether the shrimps come from fishing or farming. Only 22% of the surveyed restaurant representatives admit to pay attention on the origin of shrimps.

Fig. 106 - Restaurateurs awareness on shrimp harvesting (farming vs. fishing).

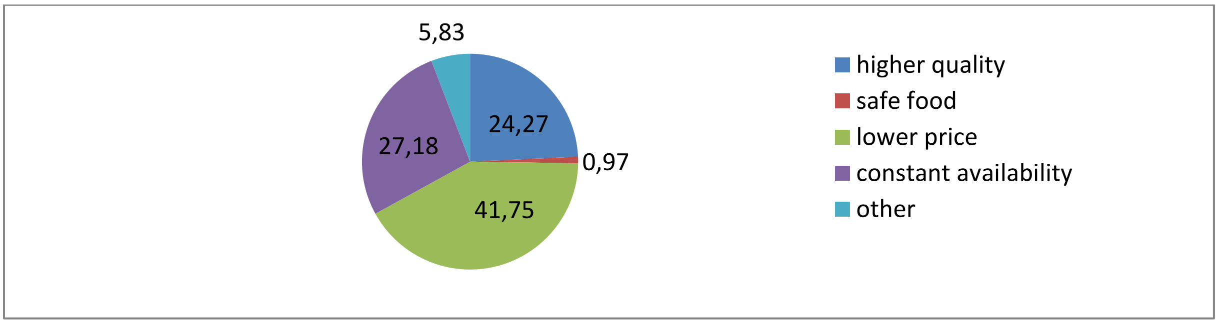


From 22% (23 out of 104 examined restaurants) paying attention on whether the shrimps come from fishing or farming, 78% choose shrimp from farms, and 22% shrimp from fishing.

Farmed shrimps – advantages and disadvantages: As the main advantage of farmed shrimps restaurants see their lower price, whereas the main disadvantage – worse taste.

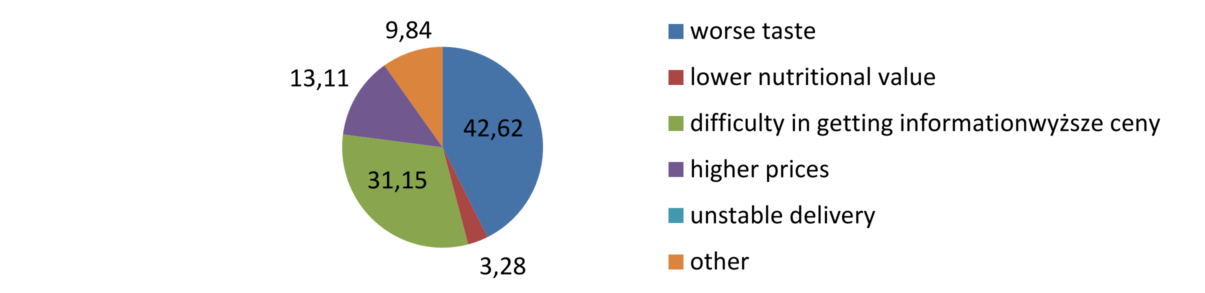
When asked about the advantages of shrimp from the farm, restaurateurs give the following answers: lower price - 42%, followed by a continuous supply - 27% and better quality - 24%.

Fig. 107 - Advantages of shrimps coming from farms according to restaurateurs



As the main disadvantage of shrimps coming from farms, restaurateurs usually point at worse taste - 43%, difficulty in obtaining information on the product- 31%, and higher prices - 13%.

Fig. 108 - disadvantages of shrimps coming from farms according to restaurateurs.



RAS shrimps: Over 60% percent of the asked restaurateurs have not heard about shrimps farmed in the recalculating aquaculture systems. Still over 80% percent of the restaurateurs is potentially interested in shrimps farmed in Poland. Question examining awareness on shrimps farmed in the reticulating aquaculture system (RAS) was asked and the answers showed that the method is not well known among the restaurateurs. Only 34% of the respondents have heard about shrimps farmed RAS systems. 66% of the respondents have not heard about the method. Interestingly, as many as 86% of restaurateurs expressed interest in supplying shrimps farmed in Poland, while 14% did not.

Metrics questions:

Questioned personnel: When examining restaurants, the answers were provided by managers (39%), chefs (29%), other personnel (18%) and owners (14%).

Respondents description:

Location: Examined restaurants are located mainly in central(49%) and tourist (44%) places. The rest of the restaurants are located away from the city center.

Type of cuisine: Examined restaurants represent mainly mixed (45%), Mediterranean (33%) and Asian (14%), sushi (7%) and Polish (2%) cuisines.

Customers: Examined restaurants are visited by middle-income clients (59%), but also by high income clients (31%), definitely less often by low income clients (10%).

General conclusion:

The analysis shows that the shrimp demand in Tri-City restaurants is low. Tiger shrimps are most frequently sold species in restaurants. The most influential factor in decision making when buying shrimps is price. Most often restaurants order large shrimps, which have been frozen and processed before purchase. Over half of the responding restaurateurs is not aware of the region of shrimps origin. For the majority of the restaurateurs the method of shrimp harvesting (fishing or farming) is also irrelevant. Still, when asked about the main advantage of farmed shrimps restaurants see their lower price, whereas the main disadvantage – worse taste. Over sixty percent of the asked restaurateurs have not heard about shrimps farmed in the recalculating aquaculture systems. Still over eighty percent of the restaurateurs is potentially interested in shrimps farmed in Poland.

Collected answers were provided by restaurants' managers, chefs, owners and other personnel. Examined restaurants are located mainly in central and tourist places and represent various cuisines.

In their menu on average over five shrimp courses are offered by a single restaurant. The average price of a main course is 46 PLN. As the interviewers admit shrimp dishes are very rarely promoted in any way. Neither do the customers ask specific questions on shrimps while ordering them.

Considering the results of the survey although shrimps demand as well as product awareness are very low, there is a potential for locally farmed shrimps, which can be sold fresh and unprocessed. It has to be kept in mind that restaurateurs are interested in large shrimps and that the price has to be competitive, since it is the most decisive factor for restaurateurs while making an order. There is also a need for investment in promotion of sustainably farmed shrimps, since restaurateurs awareness on the method of farming, specially recirculating aquaculture systems is also very low. At the same time there is also a potential in consumers, who need to be educated in sustainable and healthy farming.

4.1.2 Consumer perception (pilot)

<u>**Description of the surveys**</u>

Consumer pilot surveys

Pilot surveys were prepared and carried out in April 2018, by students of the University of Gdańsk, participants of the course: Planning research and data analysis in biological oceanography. Six questionnaires were prepared and carried out. All six questionnaires included a series of questions on Polish crustacean market, customers' preferences and awareness.

Description of respondents:

Respondents in the surveys were inhabitants of Poland. Thirty respondents took part in each of the questionnaire, one hundred eighty all together. The surveys were carried out between April and May 2018.

Conclusions from surveys:

Conclusions on crustaceans consumer structure:

Over half of the respondents consume crustaceans.

Depending on the questionnaire, the results of respondents consuming crustaceans range from 50% to 73% in the pilot questionnaires. The results of four questionnaires were considered, since four questionnaires out of six included a question about crustacean consumption. Results were the following: 50%, 73%, 61%, 62%. On the basis of four questionnaires on average 61.5% of respondents consume crustaceans. One questionnaire included a question about the purchase (not consumption) of crustaceans. The result of the survey indicated that 50% of respondents buy crustaceans.

Conclusions on frequency of crustaceans consumption:

Crustacean consumption is very rear. Four questionnaires in the pilot study included questions on the frequency of crustacean consumption. All of them proved that crustaceans are eaten very rarely. Depending on the survey, the most common answer is once a month or several times a year.

Conclusions on crustaceans purchase:

According to the answers from two surveys from the pilot study most consumers buy them in supermarkets or order in restaurants. Two questionnaires included questions on the place of crustaceans purchase.

Conclusions regarding factors affecting the purchase of crustaceans:

Survey results indicate the price as the main factor influencing the decision about the purchase. In all six pilot surveys carried out, consumers were asked about the factors affecting the purchase of crustaceans. Customers buying crustaceans admitted that they also paid attention to the taste and the product expiration date.

Conclusions regarding the most popular crustaceans consumed by respondents:

The answers show that crustaceans are the most popular among seafood, and shrimps among crustaceans. Three questionnaires in the pilot study included questions on most commonly eaten seafood/crustaceans.

Conclusions regarding consumer awareness (regional origin of crustaceans):

The received answers indicated lack of consumer awareness on this subject, only 33% of crustacean consumers replied that crustaceans came from Asian countries. A question about origin (country/region) of crustaceans was asked only in one of the pilot questionnaires prepared and surveys carried out.

Conclusions regarding consumer awareness (origin of crustaceans – farming vs. fishing):

Only half of the responding crustacean consumers draw attention to the origin of crustaceans and how they are fished or farmed. Three questionnaires included questions about the origin of crustaceans - fishing or farming. These questions differed from each other. However, the answers to the questions indicate low consumer awareness of how crustaceans are cultivated/harvested.

Conclusions regarding consumer awareness (certification):

Two questionnaires included questions on labels on the packaging of crustaceans purchased. One survey shows that over 70% of consumers read the labels of purchased crustaceans. However, at the same time, the results of the second questionnaire point to the fact that very few people are aware of the MSC certificates (labels placed on packages of fished animals).

Conclusions regarding consumer awareness (species recognition):

Three questionnaires included questions which could verify knowledge on crustacean species. Most consumers admit that they know what crustacean species are available on the Polish market, but this is the answer to the question in which consumers decide whether they know the crustacean or not. Two questionnaires contained a question with photos to match the species. The answers were very accurate. Which indicates good recognition of species available on the market.

Conclusions regarding reasons for not consuming crustaceans

Surveys show that respondents who do not consume crustaceans mainly indicate other food preferences as a reason. Their decision on lack of consumption is also related to the lack of availability of products and unprofitable prices.

General conclusions:

More than half of the respondents consume crustaceans. However, crustaceans are consumed very rarely (once a month or even less frequently). Most commonly crustaceans are purchased in supermarkets or consumed in restaurants. Price is by far the most discriminating factor for clients. Crustaceans are at the same time the most popular seafood on the market, with shrimps taking the lead. Questions regarding consumer awareness show that consumers know very little on the origin of the sea food, most of them do not know the location the crustaceans come from and only half of them pay attention to the methods of harvesting (farming vs. fishing). Still a lot of consumers admit that they read the labels on packaging, but not many are aware of the certification and its meaning. In contrary consumers are familiar with crustacean species on the market and can easily recognize the species.

Six questionnaires prepared by the students were different in structure and directed to different groups of respondents. Some of the conclusions of this report are based on all six surveys, where others only on one. Mistakes made during these pilot surveys have been taken into account and questions giving the most clear answers were unified and a questionnaire for a second consumer survey has been prepared.

4.1.3 Consumer perception

Description of the study

Consumer confirming survey

The questionnaires were prepared by the InnoAquaTech project team from the Institute of Oceanography, University of Gdańsk, based on the results of the first pilot consumer survey carried out in March 2018. The study was carried out in July 2019, through questionnaires, which were left in Tri-city restaurants serving crustaceans in their offer. The questionnaires were filled out by restaurant clients. The results form the basis of the prepared report.

Description of respondents:

The survey was carried out among 50 restaurant customers. 44% of respondents are men, and 56% are women. Respondents belong to different age groups: the largest age group 40% - are people aged 36-45, 30% - are people aged 46-60, the remaining 30% of the respondents are people aged 18 to 35. Respondents also come from different income groups. The largest group - 42% of respondents belong to the income group 2501-3500PLN gross per one person in the household, 27% of respondents belong to the income group 1501-2500 PLN, 21% from the income group 3501-4500 PLN, 6% is the income group 100-1500 PLN, and 4% - over 4500 PLN.

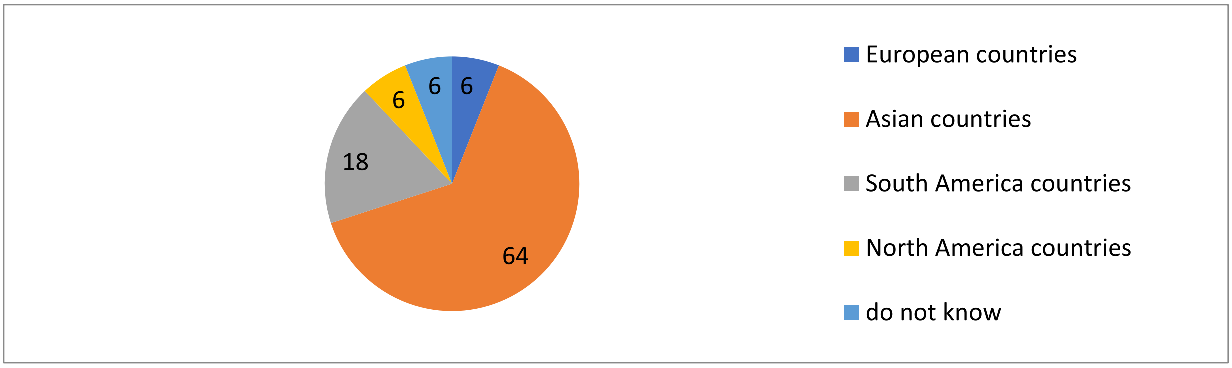
Conclusions from surveys

Consumer awareness:

Customers are aware of the regional origin of the offered crustaceans, however customer awareness in the area of the method of harvesting (fishing vs. farming) is low.

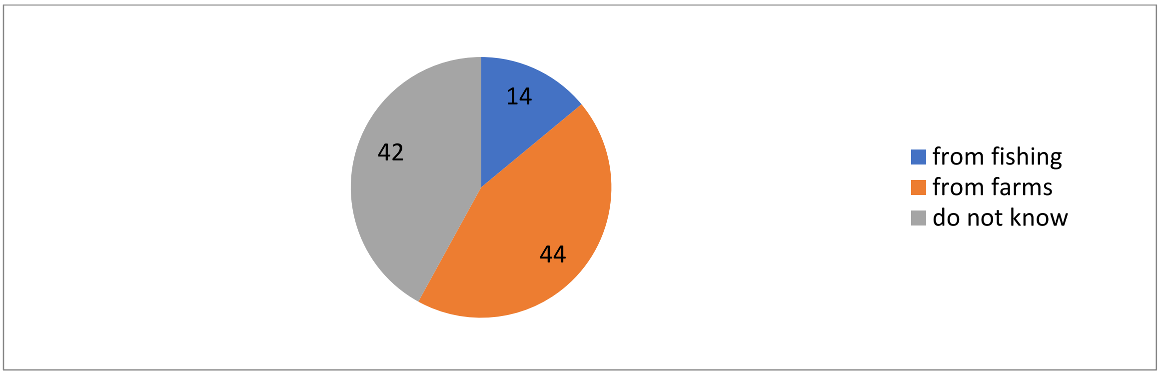
The questionnaire contains two questions about the origin of crustaceans offered on the Polish market. The first one is focused on the country/region of origin, the second one the method of harvesting farming/fishing. Answers to the first question indicate that respondents are aware of the region the majority of crustaceans offered on the Polish market come from, as up to 64% of those asked indicate Asian countries. The rest of the respondents point to the countries of South and North America, Europe or choose the answer “I do not know” (6% of respondents).

Fig. 109 - share of respondents indicating their awareness of the origin of crustaceans (country/region)



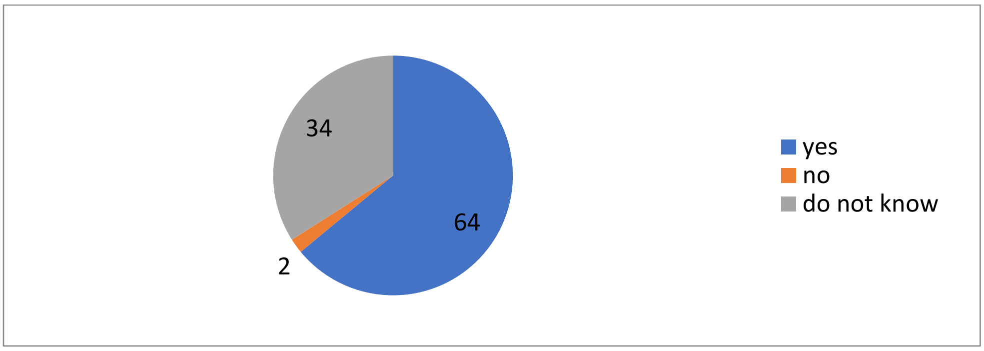
Answers to the question about the method of obtaining crustaceans offered on the Polish market (fishing/farming) indicate low consumer awareness in this respect. Only 44% of consumers answered that the majority of crustaceans comes from farms. The rest responded that crustaceans come from fishing (14%) or that they did not know (42%).

Fig. 110 - share of respondents indicating their awareness in the field of obtaining crustaceans (farming/fishing)



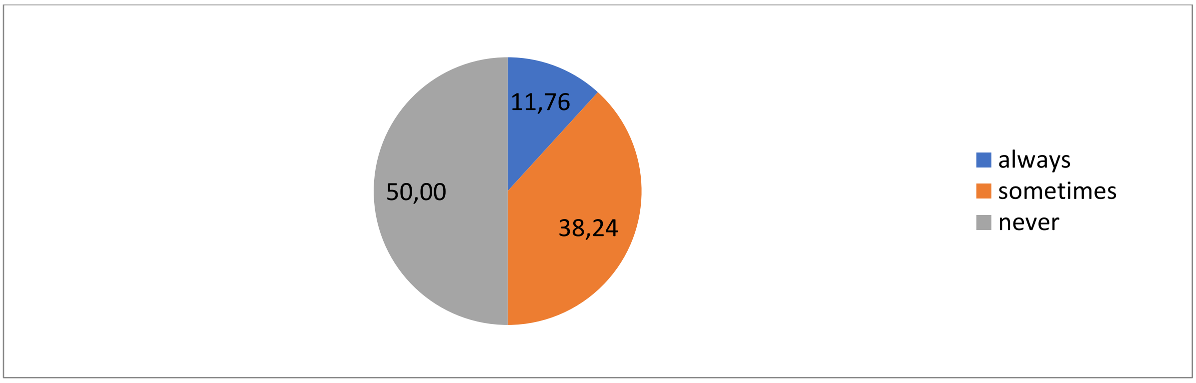
Still, when asked about the possibility of growing crustaceans in land-based cultures, and away from natural water bodies, 64% of respondents said that it was possible, 34% admitted that they did not know, and only 2% stated that it is not possible.

Fig. 111 - share of respondents indicating their awareness of the possibilities of crustaceans farming on land far from natural water reservoirs.



Product awareness among consumers consuming crustaceans is very low. 50% of consumers never read product labels. 38% read them only sometimes. Only less than 12% of respondents consuming crustaceans always read labels on the packaging of the product.

Fig. 112 - consumer awareness of the product - consumers familiarity with the product label

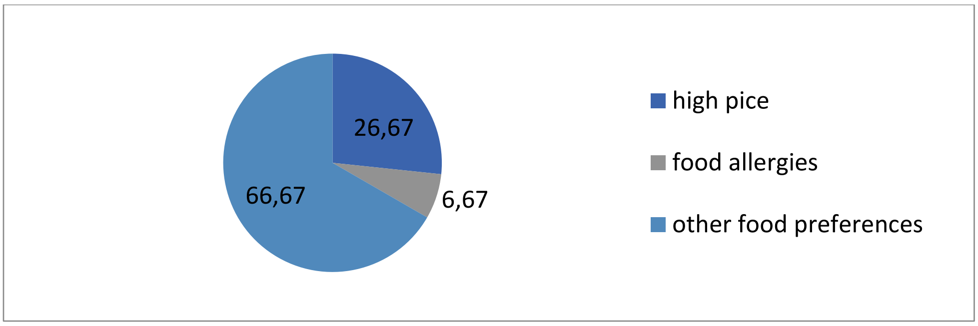


Consumption of crustaceans:

72% declared crustacean consumption. The frequency of consumption is very diverse. The decision on the purchase is most frequently based on the species of crustaceans, and shrimps are by far most popular among consumers. 28% of the respondents do not consume crustaceans. The reason for not consuming crustaceans is most commonly other food preferences.

28% of respondents who declared that they were not eating crustaceans were asked to indicate the factors that influence their decision. The factors include: high price, poor availability, food allergies, vegetarian diet, other food preferences, poor product quality, and others (to be suggested by a respondent). As many as 67% of respondents who do not consume crustaceans give "other food preferences" as the main reason for not consuming crustaceans, 27% - high price, and 7% food allergies.

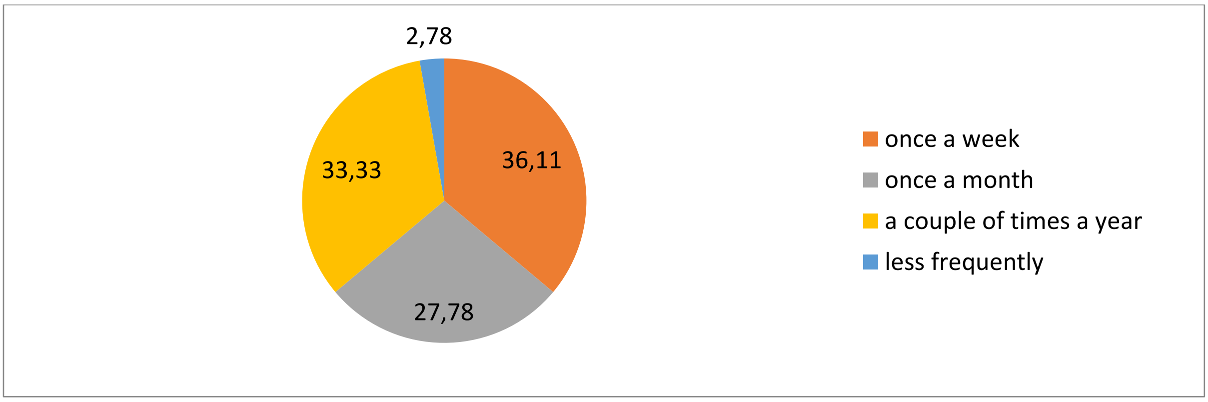
Fig. 113 - main factors determining lack of crustacean consumption



Respondents who declared consumption of crustaceans (72% of respondents) were asked about the frequency of their consumption, which crustaceans they consume most often, factors affecting the purchase of crustaceans, place of purchase and familiarity with the labels of purchased products.

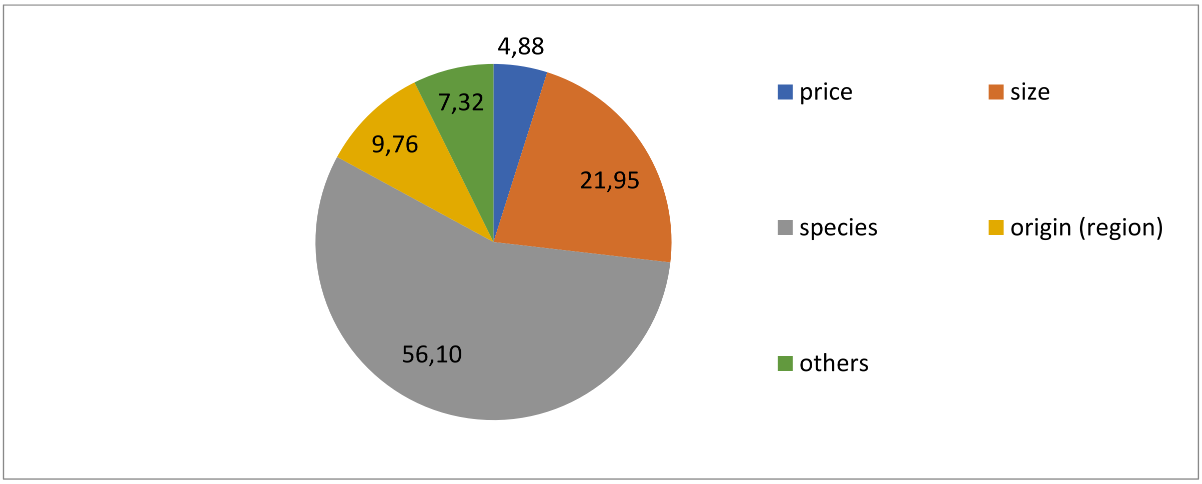
Regarding the frequency of crustacean consumption, 33% of respondents answered that they consume crustaceans several times a year, 28% - once a month and 36% - once a week. Only less than 3% of respondents consume them less than once a year.

Fig. 114 - frequency of crustacean consumption



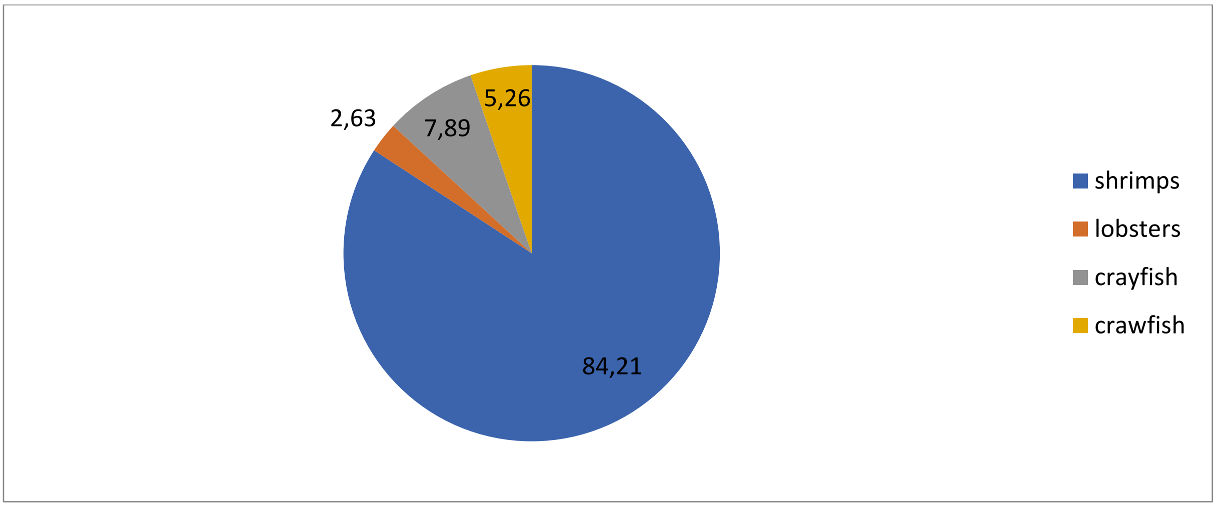
Respondents were asked about the factors that affect their decision on crustaceans purchase. Among the factors listed there are: price, size, species, area of origin, method of harvesting (farming/fishing) and other. The respondents most often refer to species – 56% of responses, the size of crustaceans –22%, the area of origin – 10% , other factors – 7%, and price – 5% .

Fig. 115 - factors influencing the purchase of crustaceans



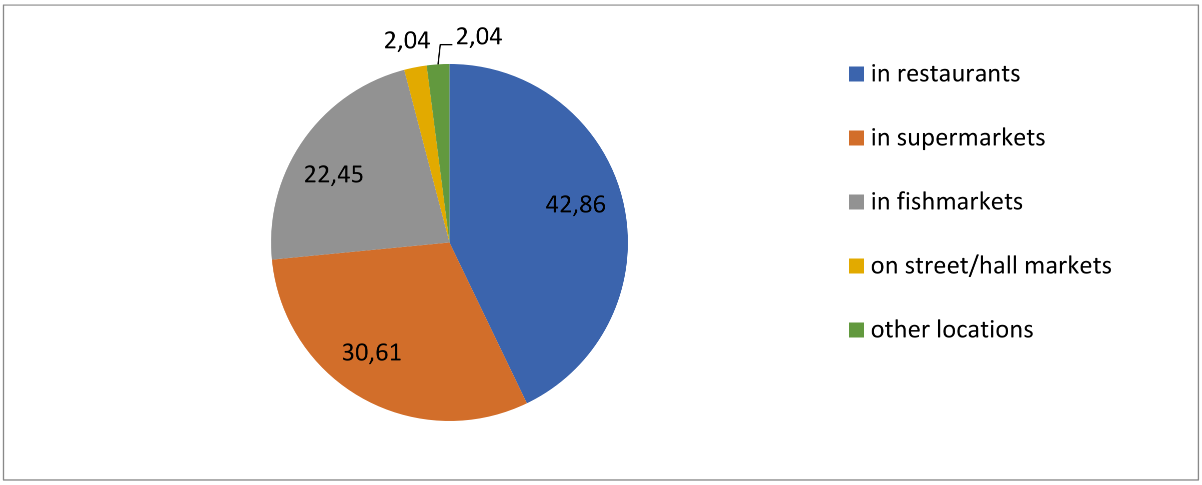
Data obtained from the answer to the question about the most frequently eaten crustaceans clearly indicate shrimps (84% of respondents consume shrimps most often). The remaining respondents (16%) are more likely to consume crayfish, crawfish and lobsters.

Fig. 116 - most frequently eaten crustaceans



Consumers most often buy crustaceans in restaurants (43%) and in supermarkets (31%). Only 22% buy them in fish markets.

Fig. 117 - consumer participation in the purchase of crustaceans in various locations



General conclusions:

In the study, 72% of respondents consume crustaceans with very different frequency. In comparison to the results of the pilot surveys the results on the consumption of crustaceans in the study are more promising and the average frequency is higher. Various factors influence their purchasing decisions, most often the respondents pay attention to the purchased species, whereas the pilot surveys showed that the most determining factor was price. Of all the crustaceans, shrimps are definitely the most popular. Most often, they are bought in restaurants and supermarkets, as the pilot studies also proved.

The respondents are aware of what region the majority of crustaceans offered on the Polish market come from, as up to 64% of those asked indicate Asian countries, where as in the pilot surveys less than half of the respondents had that knowledge. Only 44% of consumers answered that the majority of crustaceans come from farming, which is close to the result from the pilot surveys (50%). At the same time, 50% of consumers never read labels and 38% read them only sometimes. Nevertheless, most of the respondents are aware that crustaceans can be bred on land far from natural water bodies.

5. Conclusions

The attempt to cultivate whiteleg shrimps *Litopenaeus vannamei* under experimental conditions in recirculating aquaculture system (RAS) from post-larval stages to the commercial size specimens was successful: in both trials carried out at the Institute of Oceanography, University of Gdańsk the shrimps survived transport, acclimation period to laboratory conditions and reached commercial size.

It was the first experiment of whiteleg shrimp cultivation in recirculating aquaculture system Poland.

The organoleptic analysis showed that the shrimps from the experimental RAS culture achieved good quality indicators.

The analysis of elemental composition showed that the shrimps from the recirculating aquaculture system were characterized by higher level of EPA and DHA than the shrimps available on the market, allowing the placement of a nutritional health claim on a product.

There is a high interest among restaurateurs and entrepreneurs to introduce a farmed RAS shrimp into the market, and a high demand for consumer awareness raising campaigns in the area of sustainably farmed crustaceans.

Detailed conclusions on technology, species, process of farming and market are included in individual chapters.

List of annexes

1. Technology pool.

References

Archer M., 2010. Sensory assessment scoresheets for fish and shelfish – Torry and QIM. SEAFISH. Research and Development Department. https://www.seafish.org/media/Publications/sensory_assessment_scoresheets_14_5_10.pdf

Bobrański B., 1979. Analiza ilościowa związków organicznych. Wyd. Nauk. PWN. Warszawa. 328 pp (in Polish).

Burska D., 2010. Analiza elementarna (CHNS). In: Fizyczne. biologiczne i chemiczne badania morskich osadów dennych. Bolałek J. (Ed.). Wydawnictwo Uniwersytetu Gdańskiego. 291-298.

Drzazgowski J., 1995. Mikrobomba kalorymetryczna typ MI 100. Instytut Oceanografii UG. Gdynia. 13 pp (in Polish).

Gnaiger E., Bitterlich G., 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62. 289-298.

Grodzinski W., Klekowski R.Z., Duncan. A. (Eds.). 1975. Methods for Ecological Bioenergetics (Ibp Handbook, No. 24) 1st Ed. Edition. Blackwell Scientific Publications. Oxford. 367 pp.

Johnson S. K., 1995. Hand book of shrimp disease. Aquaculture, Department of Wildlife and fisheries service, Texas A and M University.

Larsson J., Smolarz K., Świeżak J., Turower M., Czerniawska N., Grahn M., 2018. Multi biomarker analysis of pollution effect on resident populations of blue mussels from the Baltic Sea. Aquatic Toxicology 198. 240-256.

Prus T., 1993. Zawartość energii w materiałach biologicznych. In: Klekowski R.Z.. Fischer Z. (Eds.). Bioenergetyka ekologiczna zwierząt zmiennocieplnych. PAN. Warszawa. 205-229 (in Polish).

Smith, P. T., 2000. Diseases of the eye of farmed shrimp *Penaeus monodon*. Diseases of Aquatic Organisms, 43:159–173

Wood E.G. (Ed.). 1997. Analytical Instrumentation Handbook. MARCEL DEKKER INC. New York. Basel. U.S.A.