

## COOPERATION AGREEMENT

**TM02000037 - Nanobubbles technology as effective tool for ozone and oxygen application in aquaculture**

(hereinafter the „Agreement”)

### Contractual Parties of the Agreement:

#### **Lead Organization**

Name: OZONTECH, s. r. o.

Id. No.: 26287781

Registered seat: K Farmě 495, 763 14 Zlín - Štípa, Czech Republic

Represented by: Martin Ševc, Executive Director

(hereinafter the “Beneficiary”)

and

#### **Project Partner No. 1**

Name: Research Institute for Aquaculture No. 1

VAT Id. No: 2300195025

Registered seat: Dinh Bang, Tu Son, Bac Ninh, 4W3R+5J, Vietnam

Represented by: Dr. Phan Thi Van, Institute Director

and

#### **Project Partner No. 2**

Name: Huy Thanh

VAT Id. No: 125010546

Registered seat: 1, Mu Cang Chai town, Mu Cang Chai, Yen Bai, Vietnam

Represented by: Nguyễn Quang Huy, Director

(hereinafter the “Partners”)

and

#### **Another Participant of the Project**

University of South Bohemia in České Budějovice

VAT Id. No: CZ60076658

Registered seat: Branišovská 1645/31a, 370 05 České Budějovice, Czech Republic

Represented by: Prof. PhDr. Bohumil Jiroušek, Dr., Rector

(hereinafter the „Another Participant”)

### **Preamble**

The Contractual Parties cooperate in the implementation of the Project No. **TM02000037** entitled “**Nanobubbles technology as effective tool for ozone and oxygen application in aquaculture** (hereinafter the “**Project**”) which the Beneficiary submitted to the 2<sup>nd</sup> public tender of the DELTA 2 support programme for applied research, experimental development and innovation (the “**Programme**”) of the Technology Agency of the Czech Republic (the „**Provider**”).

<b>Jihočeská univerzita v Českých Budějovicích</b>	
Datum: 11-06-2021	C. jednací: 7u/09/03313/21
Počet listů: 4	Počet příloh: 0

710 092100566



Supposing that the Provider enters into the Agreement on Granting of Subsidy to the Project with the Beneficiary (the “**Agreement on Granting of Subsidy**”), the Contractual Parties undertake herein to cooperate in the Project implementation and in utilizing the results of the Project.

## **Article I Subject Matter of Agreement**

- 1.1. The subject matter of this Agreement is to describe the roles and rights and obligations of the Contractual Parties relating to the implementation of the Project, in particular to define the rights and duties of the Contractual Parties with respect to (i) rights to intangible property (e.g. intellectual property) necessary for the implementation of the Project, (ii) rights to intangible property created during or in relation to the Project and (iii) regulation of utilizing the results of the Project.
- 1.2. The nature, purpose, goals and expected results of the Project are specified (i) in the Project proposal registered with the information/application system of the Provider and (ii) in the Binding parameters which form Annex 1 hereof.

## **Article II Terms and Conditions of Cooperation between Parties**

- 2.1. The Contractual Parties shall cooperate in compliance with the proposed Project and other conditions and documents that are binding for the Project. The Contractual Parties became acquainted with the Project content before signing this Agreement, including the Project application and all Programme conditions.
- 2.2. The Contractual Parties undertake to use all necessary efforts in order to achieve the purpose, goals and expected results of the Project as defined in Annex 1 to this Agreement. Failure to accomplish the purpose, goals and/or expected results of the Project may only be justified by circumstances generally recognized and defined as force majeure.
- 2.3. The Contractual Parties undertake to act and perform in a manner that will not jeopardize the implementation of the Project and the interests of the other Contractual Party.

## **Article III Structure of the Project –Investigators**

- 3.1. The person responsible for the scientific implementation of the Project by the Another Participant is the responsible investigator: [REDACTED] D., email: [REDACTED], telephone: + [REDACTED]
- 3.2. The person responsible for the scientific implementation of the Project by the Beneficiary is the principal investigator: [REDACTED], email: [REDACTED], telephone: + [REDACTED] address: K Farmě 495, 763 14 Zlín – Štípa, Czech Republic.
- 3.3. The person responsible for the scientific implementation of the Project by the Project Partner No. 1 is the responsible investigator: [REDACTED], email: [REDACTED] telephone: [REDACTED], address: Dinh Bang, Tu Son, Bac Ninh, Vietnam.

3.4. The person responsible for the scientific implementation of the Project by the Project Partner No. 2 is the responsible investigator: [REDACTED], email: [REDACTED] telephone: [REDACTED] address: 1, Mu Cang Chai town, Mu Cang Chai, Yen Bai, Vietnam.

3.5. The investigators are involved in the activities necessary for the successful completion of the Project in compliance with the approved Project proposal.

#### **Article IV**

##### **Project Management, Involvement of Individual Contractual Parties in Project**

4.1. The Beneficiary is the Project submitter and applicant for the provision of subsidy in the Czech Republic. The Beneficiary shall conclude an Agreement on Granting of Subsidy with the Provider. The Beneficiary is the coordinator of the Project and provides the administrative cooperation with the Provider in the Czech Republic.

4.2. The Partners are applicants for the provision of subsidy in the country of their origin under the terms and conditions applicable in the country where the subsidy is granted.

4.3. The Partners undertake to exercise all necessary efforts to implement the Project, and to act in a manner that will not jeopardize the implementation of the Project, the Project goals and results and the interests of the Beneficiary and Another Participant. The Beneficiary undertakes to exercise all necessary efforts to implement the Project, and to act in a manner that will not jeopardize the implementation of the Project, the Project goals and results and the interests of the Partners and Another Participant.

4.4. The Contractual Parties undertake to perform within the set deadlines and defined extent the activities leading to the Project implementation as specifically determined in the Project proposal and/or any other activities as necessary or needed for proper Project implementation.

#### **Article V**

##### **Course and Evaluation of Project**

5.1. For the purposes of verification and evaluation of progress in the Partners' cooperation during the Project implementation both Partners are obligated to provide the Beneficiary all relevant information and documents necessary to prepare:

- (a) Interim reports;
- (b) Extraordinary reports;
- (c) A final report; and
- (d) Other reports if the Beneficiary requests it.

5.2. With respect to the reports referred to in para 5.1. both Partners are obligated to adhere the instructions of the Beneficiary concerning the content and structure of the reports and deadlines for their submission and, further, to submit the reports in such a form that they could be published, as the case may be, either by the Beneficiary or the Provider.

5.3. For the purposes of verification and evaluation of progress in the Beneficiary's cooperation during the Project implementation the Beneficiary is obligated to provide the Partners reports on the Project implementation pursuant to the program conditions as required in the country where



subsidy is granted to the Partners. The Partners are obligated to inform the Beneficiary about these conditions for the submission of reports before the start of the Project implementation.

5.4. The Contractual Parties undertake to cooperate on execution of implementation plan to the Project results.

#### **Article VI Rights and Duties of Contractual Parties**

6.1. The Contractual Parties are obligated to notify each other about all changes concerning the Project, about any inability to perform obligations under this Agreement duly and in a timely manner and about all material changes and facts that could affect the implementation, expected results and goals of the Project no later than 4 calendar days from the day on which they become aware of them. The Contractual Parties are further obligated to prove at any time that they remain qualified to participate in the Project implementation.

6.2. The Contractual Parties undertake to archive documents relating to the Project for at least 10 years from the completion of the Project.

#### **Article VII Intellectual Property, Tangible Property**

7.1. This Agreement governs the rights and obligations of the Contractual Parties to intellectual property of Contracting Parties existing prior to entering into this Agreement (the "Pre-Existing Knowledge") and sets forth the rules of utilization of such Pre-Existing Knowledge for the purposes of implementation of the Project. Further, the Agreement governs the rights and obligations of the Contractual Parties to intellectual property created during the term hereof and that become the property of the Contractual Party(ies) having created it.

7.2. Intellectual property for the purposes of this Agreement means any results of intellectual activity, based on which any objectively perceivable intangible property is created. In particular, this includes inventions, technical solutions protected as a utility model, industrial designs, innovations and rationalization proposals, biotechnological inventions, trademarks, copyrighted works, know-how and other results of an intellectual activity.

7.3. Pre-Existing Knowledge which is necessary for the implementation of the Project or the utilization of its results shall remain the property of respective Contractual Party, however such Contractual Party shall permit the other Contractual Party to use any of its Pre-Existing Knowledge to the extent as necessary for the purposes of implementation of the Project.

7.4. The Contractual Parties agreed that any intellectual property created within or in connection with the Project implementation shall become the property of the Contractual Party whose employees created such intellectual property. The Contractual Parties shall notify each other of every creation of such intellectual property. The Contractual Party having rights to such intellectual property shall bear the costs of filing any applications for protection thereof and costs of relating proceedings.

7.5. If, during or in connection with the Project, any intellectual property is created as a result of mutual collaboration of employees of Contractual Party(ies) such intellectual property shall

become joint ownership of respective Contractual Party(ies), whereas the share of respective Contractual Party shall correspond to efforts exerted by each of its employees for creation of such intellectual property. The Contractual Parties shall provide mutual assistance to each other in preparation of applications, including foreign applications, for protection of such jointly owned intellectual property. The Contractual Parties shall share any and all costs of filing of applications and costs of relating proceedings in proportion of their shares.

- 7.6. If either of joint owners is not interested in filing an application for protection of intellectual property, the other joint owner(s) may request to transfer the right to file such an application to it. The joint owners will then negotiate the terms and conditions of such transfer of the right to file an application. The Contractual Parties shall provide mutual assistance to each other in preparation of applications, including foreign applications. The joint owner to which the right to file an application has been transferred bears the costs of filing of applications and costs of relating proceedings.
- 7.7. The Contractual Parties undertake to use or permit use of the Project results in accordance with their respective interests and the interests of the Provider, while respecting the necessary protection of rights to Intellectual property items and confidentiality.
- 7.8. Provided that intellectual property created during the implementation of the Project belong jointly to joint owners, the Contractual Parties undertake to use their best efforts to make an agreement regarding joint exercise of rights to jointly owned intellectual property. Consent of all joint owners is always required for (i) valid granting of a license to a third party, (ii) transfer of rights to jointly owned intellectual property to a third party and/or (iii) transfer of share to jointly owned intellectual property to a third party. Transfer of share of any of the joint owners to another joint owner does not require the consent of the others.
- 7.9. Unless agreed otherwise by joint owners, any of joint owners is entitled to transfer the intellectual property (Project result) to which is an owner or joint owner at its own expense into practice. In the case of commercial use of intellectual property by one of the Contractual Parties, the revenues from commercialization will be distributed according to the share of ownership after deducting the costs of commercialization.
- 7.10. The Contractual Parties are however entitled to use free of charge any of intellectual property created within or in connection with the Project implementation for educative, research and other non-commercial purposes.
- 7.11. Intellectual property for which the protection is possible (patents, utility models etc.) cannot be disclosed until the respective application for protection is submitted.
- 7.12. Any tangible property of Contractual Party which is necessary for the implementation of the Project or the utilization of its results shall remain the property of respective Contractual Party, however such Contractual Party shall permit the other Contractual Party to use any of its tangible property to the extent as necessary for the purposes of implementation of the Project. If, during or in connection with the Project, any tangible property is acquired or created by the Contractual Party(ies) such tangible property shall become joint ownership of respective Contractual Parties, whereas the share of respective Contractual Party shall correspond to financial means exerted by each of joint owners for acquiring or creation of such tangible property. Tangible property under this provision shall be used by any Contractual Party for the purposes of implementation of the Project.



**Article VIII**  
**Ensuring Protection of Information and Outcomes Obtained in Connection with Project**

- 8.1. The Contractual Parties undertake to provide each other with all information as necessary to carry out the activities hereunder. Unless the Contractual Parties agree otherwise all information obtained from the other Contractual Party, which is not in the public domain is considered to be confidential (the "Confidential Information").
- 8.2. The Contractual Party that has obtained such Confidential Information is obligated to maintain confidentiality thereof and ensure sufficient protection against unauthorized access. It must not disclose such Confidential Information to any other person/entity, save for its employees and other persons who are in charge of conducting activities under this Agreement and with whom the respective Contractual Party has concluded a confidentiality agreement with a scope similar to that stipulated for the Contractual Parties by this Agreement, and it shall not use the Confidential Information for any purpose other than the performance of activities under this Agreement.
- 8.3. Duties pursuant to paragraph 8.2. apply without any change and remain valid for a period of 3 years after the termination of this Agreement, notwithstanding the reason for such termination.

**Article IX**  
**Liability for Damage**

- 9.1. The Contractual Parties acknowledge that a breach of a duty under this Agreement by Contractual Party may result in incurring damage to the other Contractual Party and undertake to compensate the other Contractual Party for any caused damage.

**Article X**  
**Final Provisions**

- 10.1. This Agreement becomes valid on the date of its signature by all Contractual Parties and effective as of the signature date of the Agreement on granting of Subsidy. The Agreement is concluded for the duration of the Project and for three years after the completion of the Project. The Contractual Parties have agreed that those provisions of the Agreement which were apparently intended by the Contractual Parties to survive after the termination or expiry of the Agreement shall remain valid and effective (in particular Articles 7.1 - 7.12 and Articles 8.1 - 8.3)
- 10.2. In case of conflict between the Contractual Parties resulting from the interpretation or the application of this Agreement, or in connection with the activities contained within, the Contractual Parties involved shall make the effort to come to an amicable arrangement rapidly and in the spirit of good cooperation.
- 10.3. Failing amicable settlement, the Courts of the Czech Republic shall have sole competence to rule on any dispute between the Contractual parties in respect of this Agreement. The competent court shall be the court in the Beneficiary's seat.
- 10.4. The law applicable to this Agreement shall be the law of the Czech Republic.  
The terms and conditions of subsidy granted to the Partners by their country of origin shall be governed by valid laws and regulations of the country granting such subsidy.

10.5. The Agreement may cease to exist upon full discharge of all obligations by all Contractual Parties arising hereunder, and/or by a written agreement of the Contractual Parties in which the Contractual Parties agree upon the terms and conditions of the termination of the Agreement.

10.6. Changes and amendments to the Agreement may be made solely by agreement of the Contractual Parties in the form of written numbered amendments to the Agreement. The Partners are not entitled to transfer rights and duties hereunder to a third party without the prior written agreement of the Beneficiary.

10.7. The Agreement is made in four copies with the validity of the original, with each Contractual Party receiving one copy.

10.8. The Contractual Parties hereby declare that they have read through the whole Agreement, agree with the text, and further represent that this Agreement has been concluded in full compliance with their internal policies and that they are fully aware of the obligations they assume by concluding this Agreement.

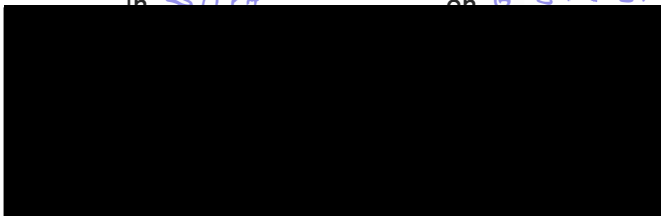
**Annexes:**

Annex 1 – Binding parameters of the Project

Annex 2 – Common proposal of the Project

**On behalf of the Beneficiary:**

In ST/PA on 6.5.2021



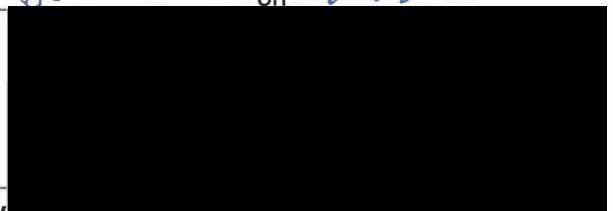
**OZONTECH, s. r. o.**

Name: Martin Ševc

Position: Executive Director

**On behalf of Another Participant:**

In C. Re on 12.5.2021

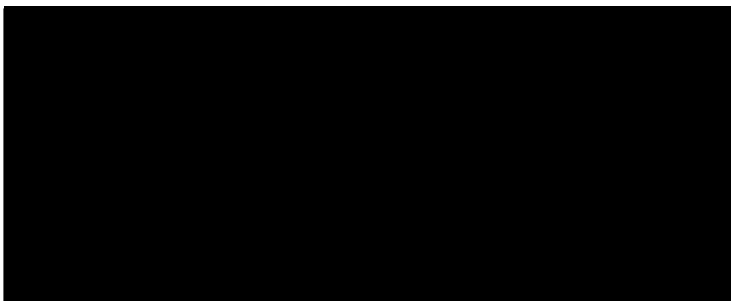


**University of South Bohemia in České Budějovice**

Name: Prof. PhDr. Bohumil Jiroušek, Dr.

Position: Rector

**On behalf of the Partner No. 1:**

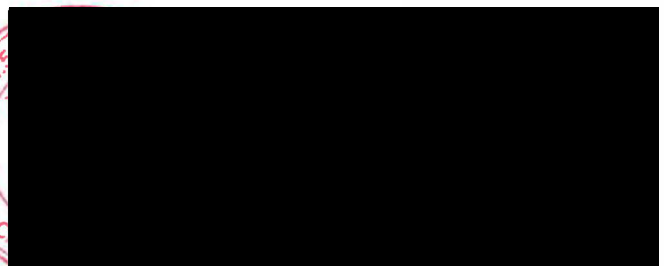


**Research Institute for Aquaculture No. 1**

Name: Dr. Phan Thi Van

Position: Institute Director

**On behalf of the Partner No. 2:**



Name: Nguyễn Quang Huy

Position: Director





## Annex 1

### BINDING PARAMETERS OF THE PROJECT

**1. The name of the Project**

Nanobubbles technology as effective tool for ozone and oxygen application in aquaculture

**2. The start date and end date of the Project**

06/2021 – 05/2024

**3. The aim of the Project**

The overall goal of the proposal is to test, implement and practically verify methods for the application of ozone and oxygen nanobubbles in aquaculture applications. Specifically, the project aims to evaluate existing knowledge on using of nanobubbles and identification of gaps in knowledge (1), develop and validate methodologies for effective application of ozone nanobubbles as treatment for disinfection during incubation of eggs of important fish species (2), improvement of water quality in aquaculture systems (3) and application of oxygen nanobubbles in intensive aquaculture systems (4). The ambition is to achieve these goals by the end of project. The goals of project are in line with the national and international priorities of supporting low-emission fish farming technologies.

**4. The Lead solver of the Project**



**5. The results of the Project**

*a) Identification number*

**TM02000037-V3**

*The name of result*

Interim report on the solution of the project

*Description of result*

Summary of current information, activities, and progress in solving the project.

*Type of result according to RIV database structure*

O – Other results

*b) Identification number*

**TM02000037-V4**

*The name of result*

Application of oxygen nanobubbles in recirculating aquaculture systems

*Description of result*

The result will be a technology describing the advantages and economic and operational demands of application of oxygen nanobubbles in recirculating aquaculture systems with the breeding of a selected species of fish. The newly presented technology using nanobubbles will be compared with the methods of oxygen application used so far. The technology will also describe the effects of the application on the health and physiology of fish and the parameters of the breeding environment.

*Type of result according to RIV database structure*

Ztech – Certified technology

*c) Identification number*

**TM02000037-V4**

*The name of result*

Application of oxygen nanobubbles in recirculating aquaculture systems

*Description of result*

The result will be a technology describing the advantages and economic and operational demands of application of oxygen nanobubbles in recirculating aquaculture systems with the breeding of a selected species of fish. The newly presented technology using nanobubbles will be compared with the methods of oxygen application used so far. The technology will also describe the effects of the application on the health and physiology of fish and the parameters of the breeding environment.

*Type of result according to RIV database structure*

Ztech – Certified technology

d) *Identification number*

**TM02000037-V5**

*The name of result*

Interim report on the solution of the project

*Description of result*

Summary of current information, activities, and progress in solving the project.

*Type of result according to RIV database structure*

O – Other results

e) *Identification number*

**TM02000037-V6**

*The name of result*

Application of ozone nanobubbles for the control of microalgae and cyanobacteria in aquaculture systems

*Description of result*

The result of the project will be an effective method of application of ozone nanobubbles in order to eliminate a to control the excessive growth of cyanobacteria and microalgae in aquaculture systems of various types (outdoor recirculation systems, hybrid multitrophic systems, intensive ponds). The current effect will be to improve the overall water quality in the system from the point of view oxygen balance, organic load, etc.). There will also be a reduction in the occurrence of substances negatively affecting the taste of produced fish (geosmin methylisoborneol).

*Type of result according to RIV database structure*

O – Other results

f) *Identification number*

**TM02000037-V7**

*The name of result*

Interim report on the solution of the project

*Description of result*

Summary of current information, activities, and progress in solving the project.

*Type of result according to RIV database structure*

O – Other results

g) *Identification number*

**TM02000037-V8**

*The name of result*

Use of ozone nanobubble application technology for egg disinfection of selected fish species.

*Description of result*

The result of the project will be a functional and highly effective method of application of ozone nanobubbles ensuring stable incubation (high hatchability) of eggs of the selected species in controlled conditions of Czech and tropical aquaculture. This will be supported by a reduction in the load of pathogens in the environment. The specific species will be selected from the group of tested species (see introduction of the project) and identified on the basis of the degree of progress achieved in the results of the application and viability of a viable fry.

*Type of result according to RIV database structure*

NmetC - Methodologies certified by an authorized body

## **6. Identification data of participants**

### **Main recipient – (P) OZONTECH, s.r.o.**

*Identification number*

26287781

*Company name*

OZONTECH, s. r. o.

*Legal form*

Legal entity registered in the Commercial Register

*Type of organization*

Small enterprise

### **Other participant – (D) – University of South Bohemia in České Budějovice**

*Identification number*

60076658

*Company name*

University of South Bohemia in České Budějovice

*Organisational unit*

Faculty of Fisheries and Protection of Waters

*Legal form*

Public university

*Type of organization*

Research organization

### **Foreign partner – (Z) – Research Institute for Aquaculture No. 1**

*VAT Identification number*

2300195025

*Company name*

Research Institute for Aquaculture No. 1

*Legal form*

Foreign state organization

*Type of organization*

Research organization

### **Foreign partner – (Z) – Huy Thanh**

*VAT Identification number*

125010546

*Company name*

Huy Thanh

*Legal form*

Foreign state organization

*Type of organization*

Small enterprise

## 7. Costs (in Czech crowns)

### Project — TM02000037

Item / Year	2021	2022	2023	2024	Total maximal amount
Total project costs	1 056 250	1 825 000	1 687 500	843 750	5 412 500
Amount of subsidy	776 250	1 343 500	1 231 500	622 250	3 973 500
Maximal % of subsidy					74 %

### Main recipient – (P) OZONTECH, s.r.o.

Item / Year	2021	2022	2023	2024	Total maximal amount
Personal costs	125 000	280 000	280 000	125 000	810 000
Subcontracting	0	0	0	0	0
Other direct costs	150 000	210 000	210 000	150 000	720 000
Indirect costs	68 750	122 500	122 500	68 750	382 500
Total project costs	343 750	612 500	612 500	343 750	1 912 500
Amount of subsidy	163 750	252 500	272 000	173 750	862 000
Method of overheads calculation					Flat rate 25 %

### Other participant – (D) – University of South Bohemia in České Budějovice

Item / Year	2021	2022	2023	2024	Total maximal amount
Personal costs	270 000	490 000	510 000	230 000	1 500 000
Subcontracting	0	0	0	0	0
Other direct costs	300 000	480 000	350 000	170 000	1 300 000
Indirect costs	142 500	242 500	215 000	100 000	700 000
Total project costs	712 500	1 212 500	1 075 000	500 000	3 500 000
Amount of subsidy	612 500	1 091 000	959 500	448 500	3 111 500
Method of overheads calculation					Flat rate 25 %

**Common Proposal**

**For TA CR-MOST Bilateral Co-funding R&D Project**

*Please note that the information provided will be taken into account when evaluating the project proposal submitted by partner institution/enterprise to the 2nd public call of the DELTA 2 programme of the Technology Agency of the Czech Republic and the Ministry of Science and Technology of Vietnam.*

*Principal Applicant/Lead Organization from the both sides shall complete this Common Proposal in collaboration and submit each to the respective agency when submitting the proposal package.*

<b>Project Title:</b> Nanobubbles technology as effective tool for ozone and oxygen application in aquaculture	
<b>Project Duration:</b> 36 months	<b>*Total Estimated Project Cost:</b> USD 550,942
<b>**Project number (on the Czech side):</b> TM02000037	<b>***Project number (on Vietnamese side):</b>

*\* Total estimated costs including the MOST and TA CR support and private resources of all participants*

*\*\* Will be generated by the TA CR information system*

*\*\*\* To be provided by the Department of International Cooperation - MOST, leave blank*

**1. Affidavit**

Organizations listed under Consortium Partners herein below (collectively referred to as the „consortium“) hereby declare and confirm as follows:

- The consortium intends to collaborate on the above-mentioned R&D project.
- No organization in the consortium is in liquidation and insolvency or impending bankruptcy, and is not being dealt with in insolvency proceedings.
- No organization in the consortium is in financial difficulty.

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## Delta 2

### Consortium Partners

<p><b>1a) Principal Applicant / Lead Organization (on the Czech side)</b> Name of institution/enterprise: OZONTECH, s.r.o. Address: K Farmě 495, 763 14 Zlín - Štípa Phone: + [REDACTED] Fax: [REDACTED] Website: <a href="https://www.ozontech.cz">https://www.ozontech.cz</a></p> <p><b>Contact Person</b> [REDACTED]</p>	<p><b>1b) Principal Applicant / Lead Organization (on the Vietnamese side)</b> Name of institution/enterprise: Research Institute for Aquaculture No. 1 Address: Dinh Bang, Tu Son, Bac Ninh Phone: [REDACTED] Fax: [REDACTED] Website: <a href="http://www.ria1.org">www.ria1.org</a></p> <p><b>Contact Person</b> [REDACTED]</p>
<p><b>2a) Participating Organization (on the Czech side)</b> Name of institution/enterprise: University of South Bohemia in České Budějovice Address: Branišovská 1645/31a, 370 05, České Budejovice Phone: [REDACTED] Fax: + [REDACTED] Website: <a href="http://www.jcu.cz">www.jcu.cz</a></p> <p><b>Contact Name:</b> [REDACTED] <b>Position:</b> Lab leader <b>Division/Department:</b> Faculty of Fisheries and Protection of Waters, Institute of Aquaculture and Protection of Waters, Institute of Aquaculture and Water Protection <b>Phone (direct):</b> [REDACTED] <b>Email:</b> [REDACTED]</p>	<p><b>2b) Participating Organization (on the Vietnamese side)</b> Name of institution/enterprise: Huy Thanh Address: 1, Mu Cang Chai town, Mu Cang Chai, Yen Bai Phone: [REDACTED] Fax: [REDACTED] Website: [REDACTED]</p> <p><b>Contact Person Name:</b> [REDACTED] <b>Division/Department:</b> Director <b>Phone (direct):</b> [REDACTED] <b>Email:</b> [REDACTED]</p>

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**Delta 2**

**2. Finance**

**1a) Total Project Budget of the Principal Applicant (on the Czech side) – USD**

Name of institution/enterprise: OZONTECH, s.r.o.

Indicator	6/2021	2022	2023	6/2024*	Total
<b>Costs</b>	13,638	24,300	24,300	13,638	75,875
<b>Support from the TA ČR</b>	6,496	10,017	10,791	6,893	34,198
<b>Private sources</b>	7,141	14,282	13,509	6,744	41,677

\* Max. project duration is 36 months.

If the government contribution does not cover all project costs, how will you finance the rest of the project costs (own company profit, bank loan, ...)? *(please specify amounts and sources of financing of the project)*

The project costs, which are not covered by project funding will be provided from own sources of OZONTECH, s.r.o company.

**1b) Declaration of the Principal Applicant (on the Vietnamese side) – USD**

Name of institution/enterprise: Research Institute for Aquaculture No. 1

Indicator	6/2021	2022	2023	6/2024*	Total
<b>Costs</b>	56,795	116,518	89,837	16,228	279,377
<b>Support from MOST</b>	44,318	104,041	77,359	16,228	241,945
<b>Institute's sources</b>	12,477	12,477	12,477		37,432

\* Max. project duration is 36 months.

If the support does not cover all project costs, how will you finance the rest of the project costs (own company profit, bank loan, ...)? *(please specify amounts and sources of financing of the project)*

**2a) Total Project Budget of the Participating Organization (on the Czech side) – USD**

Name of institution/enterprise: University of South Bohemia in České Budějovice

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## Delta 2

Indicator	6/2021	2022	2023	6/2024*	Total
Costs	28,267	48,104	42,649	19,837	138,856
Support from the TA ČR	24,300	43,283	38,066	17,793	123,443
Institute's sources	3,967	4,820	4,582	2,043	15,413

\* Max. project duration is 36 months.

If the support does not cover all project costs, how will you finance the rest of the project costs (own company profit, bank loan, ...)? *(please specify amounts and sources of financing of the project)*

The project costs, which are not covered by project funding will be provided from institutional funds of the Faculty of Fisheries and Protection of Waters (FFPW USB).

### 2b) Declaration of the Participating Organization (on the Vietnamese side) - USD

Name of institution/enterprise: Huy Thanh company

Indicator	6/2021	2022	2023	6/2024*	Total
Costs					56,834
Support from MOST			39,784		39,784
Private's sources			17,050		17,050

\* Max. project duration is 36 months.

If the support does not cover all project costs, how will you finance the rest of the project costs (own company profit, bank loan, ...)? *(please specify amounts and sources of financing of the project)*

The project costs, which are not covered by project funding will be provided from own sources of Huy Thanh company.

### 3a) a 3b) add more if necessary



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### 3. Resumé of the project

#### 1. Project Description

The overall goal of the project proposal is to test, implement and practically verify methods and procedures for the application of ozone and oxygen nanobubbles in various intensive aquaculture applications. More specifically, the project aims to evaluate existing knowledge on using of nanobubbles technology for ozone and oxygen applications and identification of gaps in our knowledge (1), develop and validate methodologies for the effective application of ozone nanobubbles as treatment for disinfection before and during incubation of eggs of economically important fish species (2), improvement of water quality in intensive aquaculture systems (3) and application of oxygen nanobubbles in intensive aquaculture systems (4). The final impact should be to increase the efficiency and volume of fish produced and strengthening of the competitiveness of the participating countries in this area. The ambition of the project is to achieve these goals by the end of the project. The mentioned goals are in line with the national and international priorities of supporting low-emission fish farming technologies.

The recent strong development of aquaculture production requires technological innovations and more efficient procedures within intensive fish farming systems. The subject of present project is to provide the necessary financial funding of material and personnel costs for performing of the experiments and activities lead to achievement of the project objectives. The aim of the first experiment/content is to clarify possibility of ozone nanobubble as safe disinfection method in hatching period. Research activities will include a series of experiments and operational verifications testing the duration of treatment, concentration (doses), and frequency of application of ozone nanobubbles during incubation of eggs in species with a long incubation period, where there is a high risk of fungal diseases. In addition, macro ozone gas will be also tested to verify the differences between macrobubble and nanobubble of oxygen in term of hatching treatment. Hatching rate, prevalence of fungal diseases, ontogenesis and frequency of deformities will be evaluated. In parallel, the water quality parameters during incubation will be evaluated. Species including rainbow trout (*Ocorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), common whitefish (*Coregonus lavaretus*), common carp (*Cyprinus carpio*) will be tested in this experiment. Among this, rainbow trout will be tested in both countries to see different between temperate and subtropical area, as the parasites can be different between these 2 areas. Common carp will be test in Vietnam. Brook trout and common whitefish will be tested in Czech Republic.

Further activities will be focused on experiments and verifications for effective improvement of water quality (hydrochemical parameters, control or elimination of

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microalgae, cyanobacteria, and pathogen in aquaculture systems). The area of testing will be procedures for the most effective application of ozone nanobubbles in terms of concentration, duration of treatment and the possibility of periodic treatment of the system. The effects of treatments on the abundance of microalgae, cyanobacteria, chlorophyll concentration and changes in hydrochemical parameters and microbiome will be evaluated. For these activities, the intensive trout culture in a RAS outdoor for market side will be tested (in Czech). Whereas, an intensive rainbow trout culture in open pond with low water discharge will be tested (in Vietnam). The microbiome in water, sediment, and gut sample of Vietnam experiment will be done in Czech. Researcher from Vietnam will be sent to Czech for obtain microbiome results.

The last area of research activities will be experiments and operational verification of oxygen application using nanobubbles technology in recirculating aquaculture systems (RAS) and their comparison with elements traditionally used for oxygen injection in RAS. The efficiency of the oxygen transfer and economic feasibility will be evaluated. For this activities, Czech partner will help Vietnam partner to build up 3 experimental RAS for research purpose. One Researcher from Vietnam will be sent to Czech for a short course study on RAS design and maintain and micobiome analysis method. One experts from Czech will visit Vietnam for help of build-up and running of RAS.

Relevant activities of the project participants will include research on the given topics, design and preparation of experiments, implementation of laboratory analyses, evaluation of data and publication of results. Thanks to the implementation of the project, there will be a reciprocal transfer of examples of "good practice" in the field of experimental development and applied operations. Aquaculture is a significant branch of animal production both in the Czech Republic and in the Socialist Republic of Vietnam (in Vietnam even more). The results of the project thus respond to the current and future needs of both countries in the form of new knowledge and procedures that will be applied.

The Czech partners of the project (the University of South Bohemia in České Budějovice and the company OZONTECH s.r.o expect the publication of one certified methodology (NmetC), two technologies (Ztech) and one result of the "other" type (except for the 3 annual reports). The Vietnamese side (Research Institute for Aquaculture No 1 and Huy Thanh company) expect the publication of three certified methodologies, one technology and 2 articles in ISI.

The composition of the consortium is a strong asset to support its ambitions. It includes top institutions and SMEs from both countries. The expertise of the partners covers the key areas essential for the successful implementation of the project. This project should lead to next and more comprehensive collaboration.

## **2. Roles of each Lead Organization and Participating Organization in both sides**

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Task no	Task Name	Description	Results
OZONTECH, s.r.o.	Construction and design of ozone nanobubble system for eggs treatment	Experimental system combined ozone generator and nanobubble generator for testing treatments on fish eggs will be constructed.	Functional system
OZONTECH, s.r.o.	Technical support during experiments	The system available will be tested, calibrated and capacity nanobubble produced will be measured during several experiments	Protocols from testing
OZONTECH, s.r.o.	Design of system for	Construction of system for ozone nanobubbles application in outside RAS system	Functional system
University of South Bohemia in České Budějovice	Project administration on Czech side	Coordination of activities on Czech side of project, organisation of meetings	Notes from meetings
University of South Bohemia in České Budějovice	Literature review	Gathering information about current state of art for nanobubbles	Review

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		technology, analyse and integrate/synthesize results of finding	
University of South Bohemia in České Budějovice	Experimental work on salmonid eggs	Design, performing and evaluation of experiments	Protocols from experiments
University of South Bohemia in České Budějovice	Experimental work on ozone nanobubbles treatment in aquaculture systems	Design, performing and evaluation of experiments	Protocols from experiments
University of South Bohemia in České Budějovice	Experimental work on oxygen nanobubble efficiency in RAS	Design, performing and evaluation of experiments	Protocols from experiments
University of South Bohemia in České Budějovice	Reporting of results	Preparation and finalisation of annual reports. Task for every year.	Submitted annual reports
University of South Bohemia in České Budějovice	Commercial-like application of ozone nanobubbles technology	Verification of methods in commercial-like conditions	Protocols and data from verification
University of South Bohemia in České Budějovice	Publication of results	Preparing manuscripts and other relevant texts	Ztech - 1x NmetC - 1x O - 4x
Research Institute for Aquaculture No1	Experimental work on ozone nanobubble for eggs treatment	Experimental system combined ozone generator and nanobubble generator for	NmetC - 1x

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		testing treatments on fish eggs will be constructed. Experiment is designed to fine the optimal ozone nanobubbles concentration, treated duration,	
Research Insitute for Aquaculture No1 and Huy Thanh Company	Experimental work on ozone nanobubbles treatment in intensive rainbow trout pond	Design, perform, and evaluation of experiments. The experiment is designed to verify the optimal treatment method (concentration, treated duration and)	NmetC - 1x
Research Insitute for Aquaculture No1 - University of South Bohemia	Experimental work on oxygen nanobubbles treatment in RAS for rainbow trout culture	Build up and test RAS. Design, perform, and evaluation of experiments. Experiment is designed to verified the optimal added concentration and optimal time. The maximum capacity of system will be verified.	Functional system Ztech - 1x NmetC - 1x

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Research Institute for Aquaculture No1 – Huy Thanh company	Preparation and finalisation of annual reports. Task for every year.	Preparing manuscripts and annual report	Reports, publications
Research Institute for Aquaculture No1	Publication of results	Preparing manuscripts and other relevant texts	

**3. Implementation Path**

The successful exploitation and commercialization of our results would benefit not only our partner companies but would also contribute to the success of aquaculture industry in both countries in general. Main possibilities of commercialization of project outputs is publication (sale) of certified methodologies and proven technologies for effective disinfection of eggs of economically important species by application of ozone nano bubbles, control and reduction of microalgae and cyanobacteria in aquaculture systems and application of oxygen nano bubbles in intensive aquaculture systems. The number of potential users for the mentioned outputs in the Czech Republic alone reaches several dozen fish farmers and other production companies. On the Vietnamese side, the aquaculture farms/companies in the North (Lao Cai, Yen Bai) and middle Vietnam (Da Lat) will be benefit from the output of this project. As the limitation of cold water in Vietnam, both quantity and quality, the improvement of incubation and rearing system (RAS or low discharge water) will help to maintain and develop number of trout farm. On the other hand, RAS give a chance for farm in other area to culture trout/sturgeon. Potential customers for project results on the Czech side include companies engaged in the incubation of salmonid fish or intensive fish farming using recirculation aquaculture systems:

**RYBÁŘSTVÍ LITOMYŠL s.r.o., Klatovské rybářství a.s., Pstruhařství ČRS Kaplice s r.o., Pstruhařství Skalní mlýn,**

**Fish producers in RAS systems or manufacturers of RAS systems:**

**Research Center for Coldwater Aquaculture (RCCA) – RIA1, Vietnam**

**Huy Thanh Company, Mu Cang Chai town, Mu Cang Chai, Yen Bai, Vietnam**



FISH Farm Bohemia s.r.o., Tilapia s.r.o., Agrico s. r. o., BioFish s.r.o., KINSKÝ Žďár a.s., EKORY Jihlava s r. o., Blatenská ryba s r.o., FISHPLANT Divize firmy - Ryby Vlček, NDCon s.r.o.,

All commercially important discoveries will be exploited using technology transfer units of the individual institutions involved.

#### 4. Basis for cooperation

The basic principles of intensive aquaculture are based on detailed knowledge of the specific requirements of different fish species for environmental parameters. These knowledges are then used to create an optimal production environment. The participating institutions have a rich history in basic and applied research in the field of aquaculture. Nanobubble technology is one of the recent innovations in intensive fish farming technology. Despite the importance and promising results from pilot studies, no project has been dedicated to this topic in Czech Republic. The implementation of the project will transfer the acquired knowledge in both countries and their application to the conditions of commercial aquaculture.

The motivation for submitting the presented project proposal is agreement and symbiosis in the research directions between the University of South Bohemia in České Budějovice and the Research Institute for Aquaculture No. 1. This cooperation has been accelerated in the last two years when in total of 6 students from Vietnam studied at the Faculty of Fisheries and Water Conservation at the University of South Bohemia.

Namely, they are Dr. Pham Thai Giang, MSc. Vu Thi Trang, Msc. Tran Quang Hung, Msc. Tuyen Van Nguyen, MSc. Kim Anh Nguyen, Bc. Tram Nguyen Thi. Further development of close collaboration of these institutions was supported by the ERASMUS + project, within which 14 mobilities were implemented in the last five years (Christoph Steinbach, Michal Hojdeckr, Vladimír Žlábek, Jáno Regenda, Tomáš Policar, Martin Prchal, Lucie Kačerová, Vlastimil Stejskal, Dang Thi Lua, Mai Van Tai, Vu Thi Trang, Pham Thai Giang, Vu Thi Hong Nguyen, Nguyen Thi La). These activities created links and generated ideas that need to be further developed through joint projects. Examples of the implementation of joint outputs (colleagues from Vietnam in bold) are given below:

Piamsomboon, P., Jaresithikunchai, J., **Hung, T.Q.**, Roytrakul, S., Wongtavatchai, J. 2020. Identification of bacterial pathogens in cultured fish with a custom peptide database

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- constructed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *BMC Vet Res* 16, 52. (IF 2018 = 1.792).
- Doan, V. H., Hoseinifar, S. H., **Hung, T.Q.**, Stejskal, V., Ringø, E., Dawood, M.A.O. Esteban, M. Á. Effects of watermelon rind powder on growth performance, innate immune response, and disease resistance of Nile tilapia (*Oreochromis niloticus*) culture under biofloc system. *Aquaculture* - <https://doi.org/10.1016/j.aquaculture.2020.735574>
- Burkina, V., Sakalli, S., **Giang, P.T.**, Grabikova, K., Stanova, A.V., Zamaratskaia, G., Zlabek, V. In Vitro Metabolic Transformation of Pharmaceuticals by Hepatic S9 Fractions from Common Carp (*Cyprinus carpio*). *Molecules*. 2019. (IF 3.060).
- Almeida AR, Jesus F, Henriques JF, Andrade TS, Barreto Â, Koba O, **Giang, P.T.**, Soares, A.M.V.M., Miguel, O., Domingues, I. The role of humic acids on gemfibrozil toxicity to zebrafish embryos. *Chemosphere*. 2018. (IF=4.427).
- Burkina, V., Zamaratskaia, G., Sakalli, S., **Giang, P.T.**, Kodes, V., Grabic, R., Velisek, J., Turek, J., Kolarova, J., Zlabek, V., Randak, T. Complex effect of pollution on fish in major rivers in the Czech Republic. *Ecotoxicology and Environmental Safety* 164: 92-99. 2018. (IF= 3.974).
- Sakalli, S., **Giang, P.T.**, Burkina, V., Zamaratskaia, G., Rasmussen, K.M., Bakal, T., Khalili T. S., Sampels, S., Kolarova, J., Grabic, R., Turek, J., Randak, T., Zlabek, V. The effects of sewage treatment plant effluents on hepatic and intestinal biomarkers in common carp (*Cyprinus carpio*). *Science of the Total Environment* 635: 1160-1169. 2018. (IF=4.9).
- Giang, P.T.**, Sakalli, S., Fedorova, G., Khalili, T.S., Bakal, T., Najmanova, L., Grabicova, K., Kolarova, J., Sampels, S., Zamaratskaia, G., Grabic, R., Randak, T., Zlabek, V., Burkina, V. Biomarker response, lipid composition, and intestinal microbiome in wild brown trout (*Salmo trutta m. fario* L.) exposed to a sewage treatment plant effluent-dominated stream. *Science of the Total Environment* 625: 1494-1509. 2018. (IF= 4.9).
- Giang, P.T.**, Burkina, V., Sakalli, S., Schmidt-Posthaus, H., Rasmussen, K.M., Randak, T., Grabic, R., Grabicova, K., Fedorova, G., Koba, O., Golovko, O., Turek, J., Cervený, D., Kolarova, J., Zlabek, V., Effects of multi-component mixtures from sewage treatment plant effluent on common carp (*Cyprinus carpio*) under fully realistic condition – a real case study. *Environmental Management*. 2017. (IF= 2.177).
- Andrade, T.S., Henriques, J.F., Almeida, A.R., Machado, A.L., Olga Koba, O., **Giang P.T.**, Soares, A.M.V.M., Domingues, I., Carbendazim exposure induces developmental, biochemical and behavioural disturbance in zebrafish embryos. *Aquatic Toxicology* 170: 390-399. 2016. (IF 2016 = 4.129).
- Burkina, V., Sakalli, S., Rasmussen, M.K., Zamaratskaia, G., Koba, O., **Giang, P.T.**, Grabic, R., Randák, T., Žlábek, V., Does dexamethasone affect hepatic CYP450 system on fish? Semi-static in-vivo experiment on juvenile rainbow trout. *Chemosphere* 139: 155-162. 2015. (IF 2014 = 3.340).



Globally, the Socialist Republic of Vietnam is one of the most important producers of aquaculture product. Research Institute for Aquaculture No. 1 (RIA1) is a leading Vietnamese aquaculture institution. For intensive fish production, the institute has developed a number of specific methodologies optimizing their breeding and rearing. The institute has high-quality instrumentation and experimental facilities as laboratories are accredited ISO 17025:2017. The laboratories are well equipped for water quality analysis and fish disease diagnostic. The main instruments include ZEE nit 700P, UV-UVisible SP-3000 Plus, nanobubble generator (Aqua+190M), Algae Lab analyser (BBE). Portfolio of RIA1 include wide network of cooperating commercial companies which are the target group for project results.

The Vietnamese partner - Huy Thanh company mainly works with cold-water aquaculture such as trout and sturgeon. The company has about 7ha in area with and numerous of tanks/ponds from 100 – 500 m<sup>2</sup> for intensive aquaculture, which fit in the project's experiment design. The company had 10 years' experience with intensive trout culture and reproduction.

#### 5. Intellectual Property Rights

The results of the development arising during the solution of the project will be distributed among the Contracting Parties in proportion to their participation in the development within the framework of the solution and the implementation of the project. The Contracting Parties shall keep records of the activities carried out, which will be the basis for the settlement of intellectual property rights. The results of the project, which will be achieved within the Project by only one Contracting Party will be wholly owned by the Contracting Party that created these results.

The results of the project, which will be achieved within the project jointly by both Contracting Parties will be jointly owned by both Contracting Parties in the

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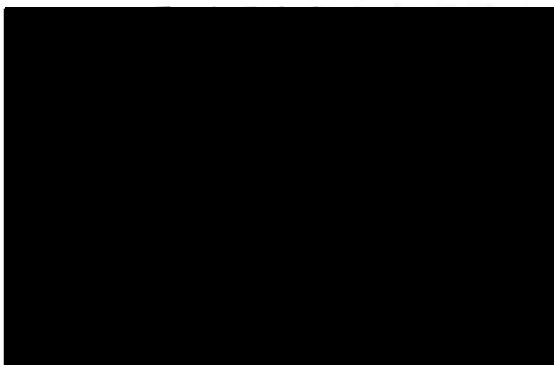
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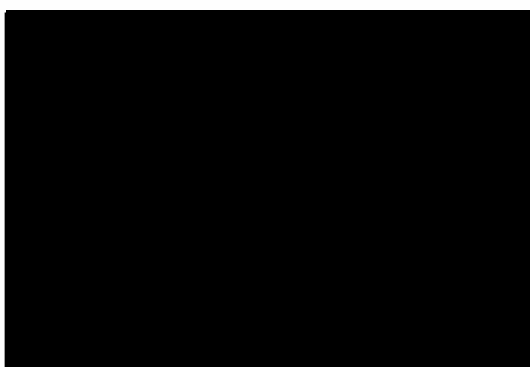
proportion in which the Contracting Parties participated in the solution and implementation of the part of the project in question. The contracting parties are entitled to protect their intellectual property rights in the usual way, provided that they have the right to publish or use partial results of their own activities for their own activities. In the case of conducting proceedings for the protection of intellectual property before the Industrial Property Office, the costs associated to the protection and proceedings before the Office are divided according to the inventor's share of the result between the Contracting Parties.

4. Statutory representative/member of a statutory body/representative with authorization to sign on behalf of the organization (according to the commercial or other register).

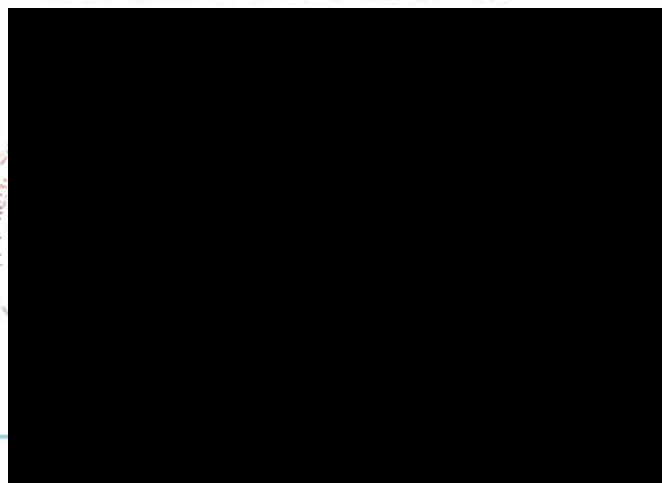
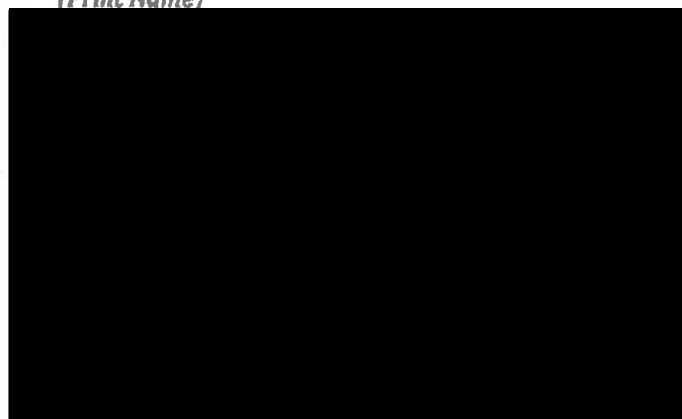
(signature) (Date)  
Principal Applicant on the Czech side



(signature) (Date)  
Participation Organization  
on the Czech side 15.04.2020  
(Print Name)



(signature and stamp)  
(Date)  
on behalf of the lead organization on the  
Vietnamese side  
(Print Name)



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