



## ECOPRO COLD Tech Sheet

ECOPRO COLD is a blend of selected strains of beneficial bacteria at a total cell concentration of  $3 \times 10^{11}$  cels/kg, a 100 % organic complete balanced nutrient formula to multiply the strains of ECOPRO and an organic chlorine neutralizer.

ECOPRO COLD works at temperatures of 3 to 25°C.

ECOPOR COLD works through diverse mechanisms:

- Breaks down particulate organic matter by releasing exoenzymes
- Breaks down mucopolysaccharides, produced by Gram negative bacteria, that form a physical barrier to oxygen and lead to anaerobic sediments
- Absorbs dissolved organic matter more efficiently than Gram negative bacteria
- Absorbs ammonia and nitrites
- Reduces populations of deleterious bacteria by competition for nutrients and by production of active metabolites
- Enhances production of digestive enzymes (amylase, lipase, trypsin) in the digestive system of the cultured animals, leading to improve feed and protein conversion efficiencies that reduce feeding costs and increase growth and overhaul production
- Reduces or eliminates water exchange requirements saving energy and reducing risk of bringing unwanted microbes into the system

## Application procedure

In order to obtain the benefits from probiotic strains we require both: the appropriate strains of bacteria, and the number of cells to carry out the activity. In order to get the numbers of cells to efficiently carry out the cleaning process at low cost we need to multiply the cells of ECOPRO COLD.

### Procedure to incubate ECOPRO COLD

Wash a plastic tub and lid with dish soap, rinse well, fill recipient with water, add commercial liquid bleach (table presented below for determination of volume of sodium hypochlorite at different commercial concentrations needed per liter of water) or calcium hypochlorite in powder to get a final chlorine concentration of 10 ppm and leave for one hour. If you have an air pump, place the air-stone hooked to the pump in the water and turn it on during the disinfection process. Filter the air (0.2 microns) if you have the equipment.

	5.00%	6.00%	7.00%	8.00%
	0.42	0.35	0.3	0.26

After the disinfection process dissolve ECOPRO COLD in the water at a rate of 10 grams per liter, leave the air pump on, place the lid and leave incubating for 18 to 24 hours at 12 to 25° C. At the end of the incubation period the number of bacteria should have multiplied between 300 and 600 times, depending on the water quality, temperature and rate of aeration (amount of air and bubble size).

After the incubation process, pour the content of the tub directly into the water of the rearing tank or pond. If a paddle wheel aerator is used, we recommend to add the product in front of the created current to facilitate spread the product throughout the rearing system. Add the product after adding water to the system. The dose rate and frequency of application depend on the stocking density and water quality.

### Dosage rate

Doses are presented in grams of ECOPRO COLD used to prepare the product per cubic meter of culture water. The volume of freshly brewed ECOPRO COLD to be added per cubic meter of culture water, prepared at a concentration of 10 grams per liter, is presented between brackets.

## Crustaceans – Shrimp

### Larval cycle

Stage of development	Dose of ECOPRO mg/ m <sup>3</sup> (ml brew/ m <sup>3</sup> )
Zoea 1	15 (1.5)
Zoea 2	20 (2)
Zoea 3	25 (2.5)

### Growth cycle in ponds

Type of system	Stocking Density (PL/m <sup>2</sup> )	Dose ECOPRO (gr/ha/week)	Number of applications per week	Dose per application gr/ha/application (L brew/ ha/application)
Extensive	4 – 10	110	1	110 (11)
Semi-intensive	10 – 30	270	3	90 (9)
Intensive	30 - 300	350	3.5 (every other day)	100 (10)
Super-intensive	300 – 450	490	7 (daily)	70 (7)

- Under conditions of poor water quality due to excess organic load, or high Vibrio densities (> 2000 UFC/ml), we recommend to add daily 10 ml/ m<sup>3</sup> of freshly brewed ECOPRO until satisfactory conditions are reached.

For the intensive culture of shrimp in bioreactors or heavily aerated ponds we recommend the application of daily dose rates from 40 to 220 mg of ECOPRO per m<sup>3</sup>.

## Vertebrates – Fish

### Larval cycle

	Dose of ECOPRO mg/ m <sup>3</sup> (ml brew/ m <sup>3</sup> )
Tank preparation	40 (4)
Maintenance every 3 days	30 - 90 (3 - 9)

### Growth cycle

#### 1. Initial application

We recommend to start with a high dose application. Differences in production systems, stocking densities, and water composition lead to a wide range of dosage rates.

- After filling pond: 30 mg/ m<sup>3</sup> (3 ml brew /m<sup>3</sup>)
- After pond fertilization: 40 mg/ m<sup>3</sup> (4 ml brew /m<sup>3</sup>)
- If treatment starts in ongoing production cycle: add from 30 to 40 mg/ m<sup>3</sup> (3 to 4 ml brew /m<sup>3</sup>), depending on the organic load

#### 2. Maintenance program

- In closed systems you may add the weekly dose in one application.
- In flow-through systems we recommend to divide the weekly dose in several applications to keep a base concentration in the system.
- Add weekly 40 mg/ m<sup>3</sup> (4 ml brew/ m<sup>3</sup>) for a target biomass of fish of 10 kg/m<sup>3</sup>.

- Under conditions of high organic load or intensive fish culture we recommend the use of 80 to 180 mg/ m<sup>3</sup> daily (8 to 18 ml brew/ m<sup>3</sup>).
3. Control of deleterious microbes in culture system
- Brew 70 to 180 mg of ECOPRO per cubic meter of the production system, add this product daily for three days to a week.

## Recommendations

Do not skip the brewing process and add the probiotic directly to the production system. The rich nutrient formula might feed deleterious microbes that might be present in large concentrations in the production system.

Use all the product that has been incubated. Do not store left over product. Do not attempt to add nutrients and continue multiplying the cells as changes in the proportion of cells and in species composition (contamination) will lead to ineffective and sometimes deleterious brews that may cause mortality in your production system.