Hatchery techniques for the seed production of short-necked clams (*Paphia undulata*) in Nha Trang, Vietnam

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Induced spawning via a wet-stimulus thermal shock.

Aquaculture in Vietnam is of great importance due to its contribution to food security, employment, and economic value to the country. In fact, Vietnam is currently ranked as the fourth-largest seafood producer after China, India, and Indonesia, with an aquaculture production of 3.82 million tonnes in 2017 (FAO, 2018). With a long coastline of 3,260 kilometres and various islands and bays (FAO, 2019), Vietnam has great geographic potential for aquaculture growth.

Asia is the main producer of marine and coastal molluscs worldwide with an aquaculture production of 15.55 million tonnes, which is over 92% of the world's production in 2016 (FAO, 2018). One mollusc species, *Paphia undulata* (Born 1778), has become a popular food in most Asian countries, and its cultivation has been grown in popularity due to the depletion of its natural population and high economic value (Norte-Campos, 2010).

The short-necked clam *P. undulata* is a commercially important bivalve species with smooth and glossy, oval-shaped shells. They thrive in muddy (70%), sand or silt (30%) bottoms, with environmental conditions at a salinity of 30 to 35 ppt, dissolved oxygen greater than 4 milligrams per litre, and a pH range of 7.5 to 8.2.

Induced spawning via dry-stimulus thermal shock.

P. undulata hatchery history in Vietnam

Aquaculture of short-necked clam *P. undulata* in Vietnam started approximately ten years ago, but production has been very small due to the lack of seed supply from hatcheries. Famers either have to collect seed from natural sets (Helm, Bourne & Lovatelli, 2004) or import them from China, both of which result in extra production cost and unreliable supply to meet grow out culture requirements. As such, the potential of *P. undulata* as a new species for Vietnamese aquaculture remains unrealised, despite having started a long time ago. Novel seed production techniques are being researched and practiced at our mollusc hatchery in Nha Trang, Vietnam, to meet the market demand of *P. undulata*.

Despite having a large area for bivalve grow-out culture and being geographically close to the Chinese border to facilitate exports, Quang Ninh province, in Northern Vietnam, is not suitable for artificial seed production of aquatic species due to its high seasonal fluctuations in temperature. On the other hand, Khanh Hoa province, in Central Vietnam, has more stable environmental conditions and is better suited for seed production. Furthermore, our hatchery, being a part of the Institute of Aquaculture at Nha Trang University, is the first to conduct artificial seed production research on *P. undulata* and is, therefore, the first hatchery in Khanh Hoa province to produce seed of this species.

Broodstock preparation

Due to the close proximity of the hatchery to the sea (around one kilometre), water can be pumped directly from the marine environment to mimic natural conditions. Water is treated with chlorine at 10 ppm to reduce total suspended solids (TSS) in water for healthier seed production and is allowed to settle in tanks for three days before being pumped to spawning and rearing tanks.

P. undulata has a continuous breeding season, but its peak spawning is from July to December and is determined by food availability in the environment (Dai, 2019). Broodstock is selected directly from the wild population during spawning season with criteria for selection being over one year old (1+), having a minimum shell length of 5.5 centimetres, and weight 40 to 50 individuals per kilogram. Five kilograms, or around 200 individual broodstock, are kept in tanks with similar environmental parameters as previously described to acclimatise for at least three days and are fed 500,000 cells

Algal culture, Chaetoceros calcitrans.

per millilitre of *Chaetoceros calcitrans* @40 litres a day, which is 10 times higher than the amount of algae they will receive during rearing.

After acclimatisation, the clams are checked for maturity, as well as gonadal development stages. Sexual maturity of *P. undulata* can be determined by the size of the meat and the colour of the gonads. Mature individuals have gonads that are milky in colour. The clams can also be identified as male or female based on the viscosity of the gametes. When the gonads are gently cut open, sperm has a higher viscosity than eggs. If at least 70% of the broodstock is determined to be sexually mature, artificial spawning will be induced, otherwise they will continue to be fed with a high density of *C. calcitrans* and rechecked for sexual maturity every seven days.

Induced spawning

To induce spawning in *P. undulata*, the common practice is to inject spawning agents such as serotonin, potassium chloride, and other chemicals into the muscular foot of the clams (Salleh, 1989; Phongthana, 1993; Aguilar, 2001), but chemicals have adverse effects on both seed quality and viability (Helm, Bourne & Lovatelli, 2004). Therefore, instead of chemicals, the less invasive and stressful technique of thermal shock is used with the novel combination of dry and wet stimuli.

First, the broodstock is removed and drained from rearing tanks where the water temperature is maintained at 25 to 26 degrees Celsius. They are covered with wet sponges to avoid desiccation and are placed under the sun in their holding baskets for 30 minutes to raise their internal temperature to approximately 28 degrees Celsius. Next, water shock is induced by placing them in a container with 40 degrees Celsius water for 5 minutes. Finally, they will be hung over the spawning tanks, which are 10.5 cubic metres in volume (3.5 × 2.0 × 1.5 m), and allowed to spawn overnight.

The male clams will respond to the thermal shock first and release spermatozoa through their two siphons, which induces the female clams to release their eggs. The eggs are naturally fertilised in the spawning tanks. The number of gametes spawned using this method is dependent on the maturity level of the broodstock, but five kilograms of *P. undulata* can fill five spawning tanks at a density of five fertilised eggs per millilitre. Embryonic development will be completed and reach D-shaped larval stage after sixteen hours.

Larval rearing

D-shaped larvae spend approximately 10 to 14 days in the veliger stage dependent on temperature. When the larvae change into post-umbo larvae with hinge appearance and a size of approximately 200 to 300 micrometres in length, they are transferred, using mesh nets, to shorter tanks (8.5 × 2.0 × 0.6 m) at a density of six individuals per square centimetre to facilitate feeding. During rearing, the larvae are fed 20 litres of *C. calcitran* at 30,000 cells per millilitre twice a day. The tanks are aerated using air stones with two stones in the spawning tanks and eight stones in the short tanks.

Hatchery to open sea

The larvae will spend at least 50 days in the short tanks before reaching 2 to 3 millimetres in length, which is the suitable size for grow-out culture. At this point, the seed are sold to farmers in Quang Ninh province, in Northern Vietnam, due to its geographic advantages for bivalve aquaculture as previously mentioned.

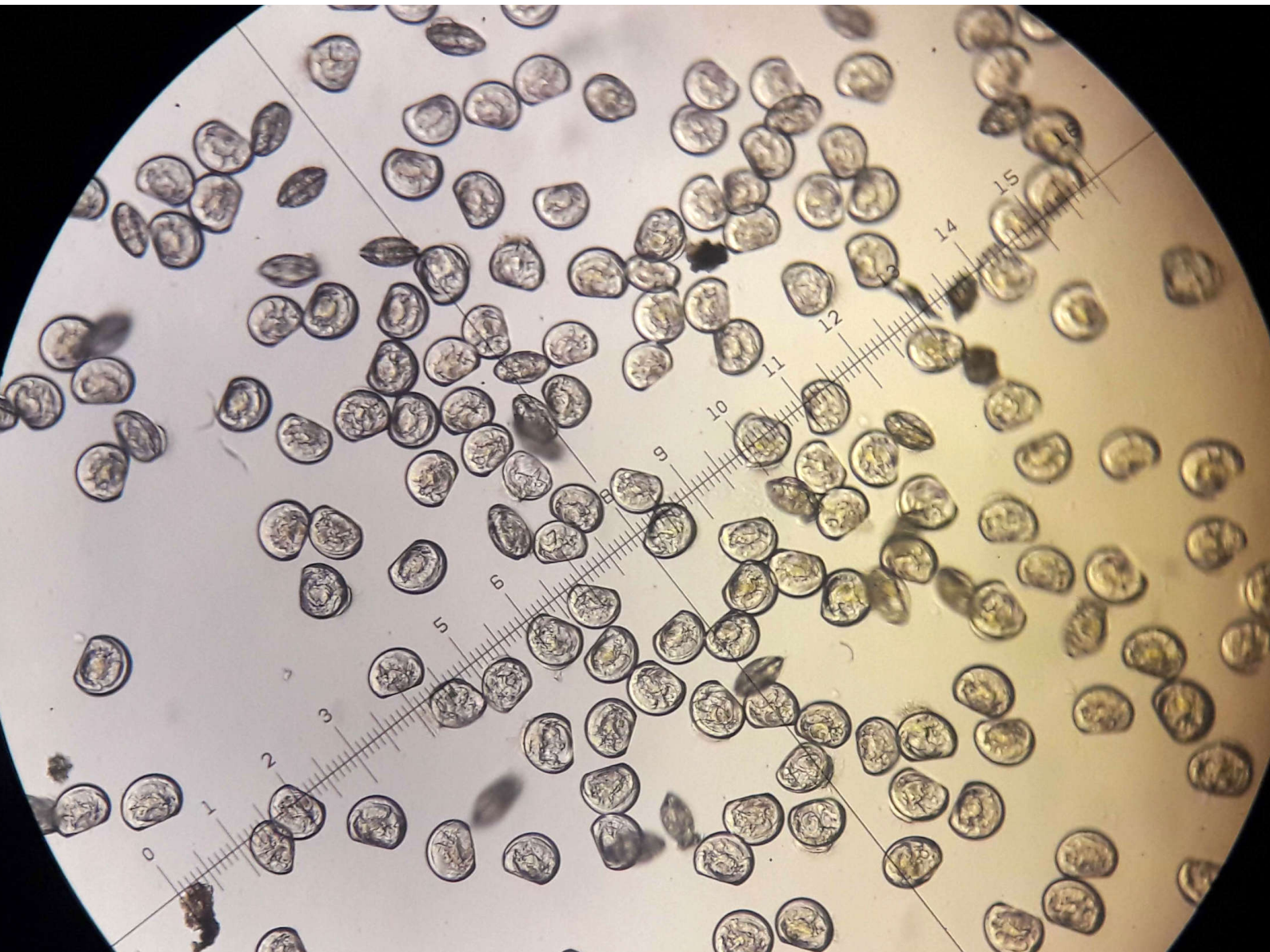
The seed are sent to the farmers in small plastic bags (40 × 20 cm), at a density of approximately 11,000 seed per bag, filled with clean seawater and pure oxygen. Seed are not fed for at least 24 hours before transfer to enhance their survival rates. The water temperature is lowered to 24 to 25 degrees Celsius to induce the juveniles into dormant states. The plastic bags are then placed into a large styrofoam box, which can hold sixteen plastic bags, with two bottles of ice to maintain a stable internal temperature of 24 to 25 degrees Celsius during the long transport from Nha Trang to Quang Ninh via air and land transfers, which can take up to 16 hours.

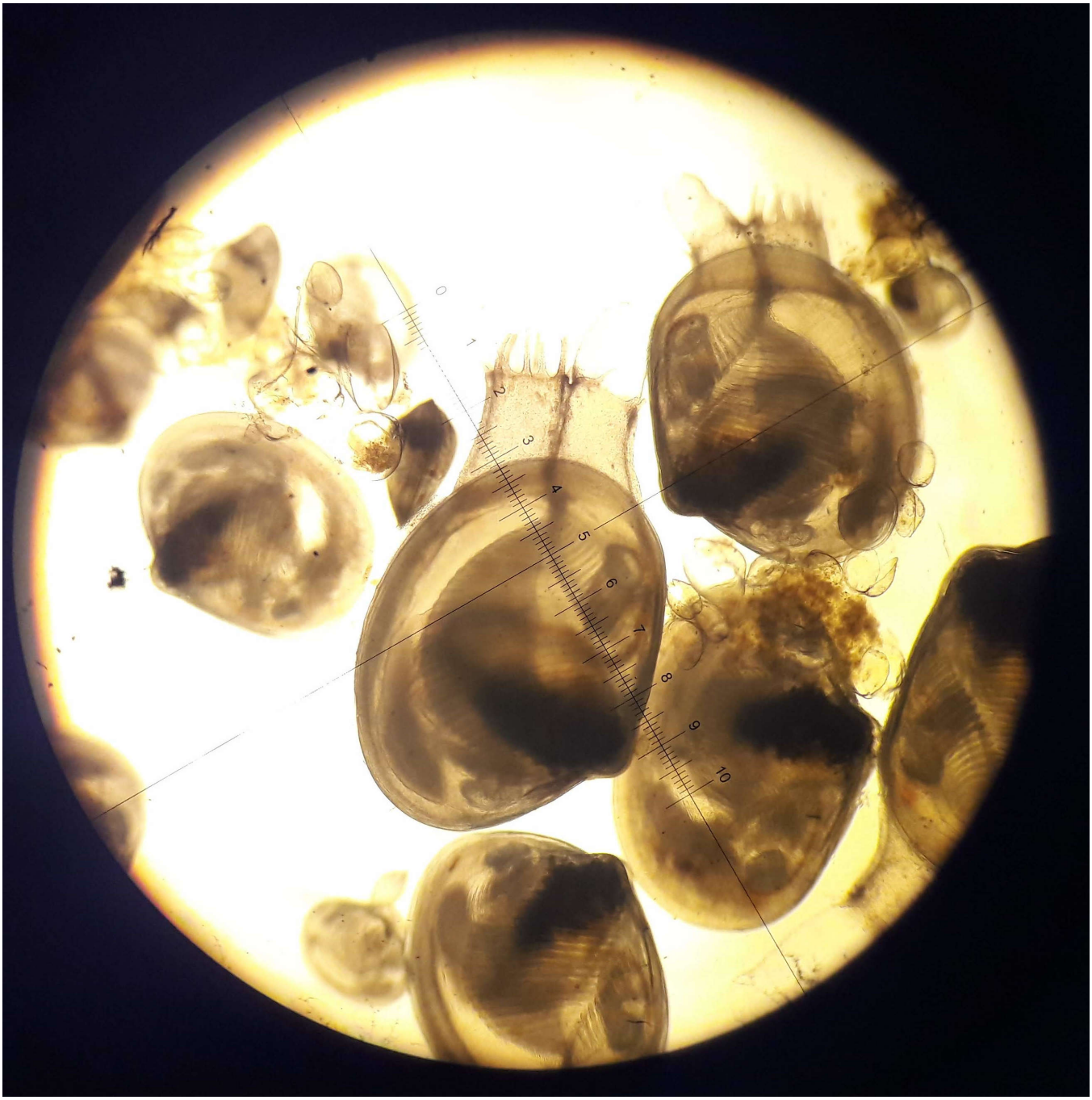
Before planting the clams, farmers have to apply for a license to allow for the cultivation of *P. undulata* in the sea. Then they will have to select and prepare a clean and suitable bottom with appropriate composition, as described above, which has to be at least 4 meters in depth, for planting. After these preparations, the seed can either be planted directly at the bottom of the sea at a density of 1,000 individuals per square metre or planted into baskets (60 × 45 × 25 cm) first, at a density of 2,000 individuals per basket, then planted on the seafloor. These *P. undulata* juveniles will reach a marketable size of 4.5 to 5.5 centimetres in length and are harvested after ten months.

Market value

The price of *P. undulata* seed is 20 Vietnamese dong per individual while the price of clams at marketable size of 70 to 80 individuals per kilogram can fetch approximately 120,000

D-shaped larvae.





Spat.

Vietnamese dong per kilogram, and are highly valued due to their delicious meat and low cost. Factoring in a survival rate of 40% from seed size of 3 millimetres to marketable size due to environmental fluctuations at sea throughout the year, *P. undulata* from seed to harvest presents farmers with an approximately 30 to 35 times return on their investment and effort.

Presently, China is the biggest importer of *P. undulata* from Vietnam, purchasing all yield from aquaculture in Quang Ninh province and 60% of total yield from fisheries in Central Vietnam. The remaining 40% of *P. undulata* caught from fisheries are being supplied to domestic markets such as restaurants.

Due to market demand and value, as well as low production cost, artificial production research on *P. undulata* is needed and should be continued in order to produce high-quality seeds to meet the market demand and to ease fisheries pressure on this species to allow the wild population to recover.

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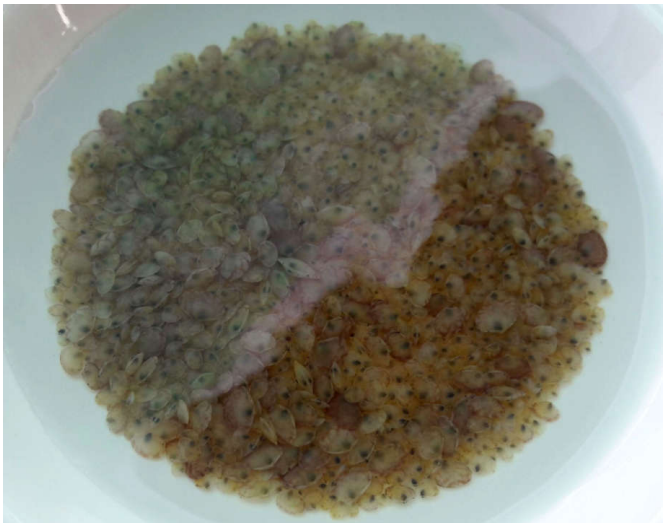

Phapia undulata seed.

Short tanks.

Long tanks.