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ScienceDirect
Aquaculture
Aquaculture 260 (2006) 181 – 193
www.elsevier.com/locate/aqua-online

A soft technology to improve survival and reproductive performance of *Litopenaeus stylirostris* by counterbalancing physiological disturbances associated with handling stress

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Received 2 December 2005; received in revised form 15 June 2006; accepted 21 June 2006

Abstract

The consequences of handling stress (fishing, transfer, eyestalk ablation) on shrimp broodstock are poorly documented. The weakness of farmed shrimp, *Litopenaeus stylirostris*, during winter is a major problem in New Caledonia, because of seasonal climate (tropical–sub-temperate). The transfer of broodstock in winter from earthen outdoor ponds to indoor maturation tanks in the hatchery (T=20 °C, Salinity=35‰, fed shrimp) usually leads, after 48 h, to high mortality (up to 70%). Eyestalk ablation to induce ovarian maturation in females leads to further mortality.

Starting from a background analysis of physiological disturbances (initial osmoregulatory imbalance) associated with handling stress (Wabete, N., Chim, L., Lemaire, P., Massabuau, J.-C., 2004. Caractérisation de problèmes de physiologie respiratoire et d'échanges ioniques associés à la manipulation chez la crevette pénéide *Litopenaeus stylirostris* à 20 °C. Styli 2003. Trente ans de crevetticulture en Nouvelle-Calédonie. Ed. Ifremer. Actes Colloq. 38, 75-84.), we developed a protocol using a soft technology, based on modifications of water salinity, temperature and feeding regime. The aim was to minimize problems of osmoregulatory imbalance and associated mortalities. The protocol we developed, called the LSD OT protocol (Low Salinity and Diet, Optimal Temperature), was first evaluated on sub-adult shrimp (20–25 g) and then applied to broodstock. Survival after transfer and following eyestalk ablation, as well as reproductive achievement (spawning rate, nauplii number) was considerably improved when shrimps were transferred under "physiological comfort" i.e. warmed isosmotic water (26 °C and 26‰) and unfed for 3 d. This new handling protocol, based on a better control of salinity, temperature and feeding conditions, has been transferred successfully to private hatcheries and already contributes to an increased profitability of New-Caledonian shrimp industry.

Keywords: Shrimp; *Litopenaeus stylirostris*; Broodstock; Handling stress; Spawning; Eyestalk ablation

1. Introduction

In 2004, New Caledonia produced 2200 tonnes of top quality farmed shrimps. The whole Caledonian production

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doi:10.1016/j.aquaculture.2006.06.041

is based on a single species, *Litopenaeus stylirostris*, endemic to the Pacific coasts of Central and South America (from Mexico to northern Peru) (Pérez Farfante and Kensley, 1997). The control of the complete life cycle (AQUACOP, 1979) and the development of specific rearing techniques (AQUACOP, 1983; Galinié, 1989) have allowed this introduced species' commercial farming since 1980.

After being reared in earthen ponds, broodstock are harvested and transferred to the hatchery where they are conditioned for reproduction (Galinié, 1989). The handling during transfer and eyestalk ablation of the females cause a high mortality rate, observed in all the hatcheries both in cold and hot seasons. Consequently, 20 000 broodstock shrimps (males and females) must be reared to obtain the 200 million post-larvae necessary to stock in the grow-out ponds.

Eyestalk ablation is carried out a few days after the shrimps' stocking into the hatchery, to induce ovarian maturation and spawning (Chamberlain and Lawrence, 1981; Ottogalli et al., 1988). Each eyestalk contains a neuroendocrine complex that synthesises and secretes the Gonado Inhibiting Hormone (GIH) (Charniaux-Cotton, 1985). The removal of one source of GIH is sufficient to prevent the inhibition of vitellogenesis and trigger ovarian maturation. This ablation is obviously a stressful factor which affects many aspects of the animal's physiology including the immune system (Perazzolo et al., 2002) and can alter the survival of the broodstock (Browdy, 1992). In the shrimp *Penaeus notialis*, Rosas et al. (1993) reported that the stress caused by unilateral eyestalk ablation led to an increase in ventilatory activity and a decrease in oxidative metabolism.

The metabolic and physiological effects of the handling of crustaceans have been less studied. In *L. stylirostris,* osmotic pressure approximately equals 810 mosM kg^{-1} when water temperatures are between 20 and 28 °C. Handling stress of the animal during transfer into a hatchery leads to disturbances in osmoregulation and oxygen transport from the gills to the tissues. In particular, a decrease in oxygen affinity of hemocyanin caused by an increase in osmotic blood pressure has been demonstrated, whereas partial oxygen pressure in the arterial blood remains stable. It has also been shown that shrimp transferred at 25–28 °C requires 24 h to recover its osmoregulatory capacity (OC), while in winter, at 22 °C, recovery time can be as long as 5 to 7 d (Wabete et al., 2004). These disturbances are associated with problems of oxygen supply which weaken the animals and could ultimately result in a heart attack. A similar result has been described in the case of acid rain toxicity mechanisms and hydromineral disturbances induced in fish (Wood, 1989).

In this study, based on our analysis of ionoregulation problems caused by the handling of *L. stylirostris* (Wabete et al., 2004), we have perfected a protocol using a "soft" technology which could reduce the results of handling stress and the associated mortality. Our approach was based on adjustments to salinity, temperature and feeding regime. The protocol was tested in New Caledonia on sub-adult shrimps and broodstock in experimental and commercial hatcheries. The transfer conditions were evaluated according to the survival and the reproductive achievement of the broodstock: maturation of the females, fecundity and number of nauplii per spawn. As the problems associated with handling began with an imbalance in the ion exchange system, the idea was: (i) to manipulate water salinity in order to limit ion and water exchanges and the associated variations in blood pH (Truchot, 1987); (ii) to promote the functioning of ion exchange systems by returning to the optimal temperature for the species; (iii) to avoid feeding shrimps in order to limit oxygen requirements, to minimize use of gas transport system and transbranchial ion exchanges which ensure the acid–base regulation in the blood during post-prandial period (Legeay and Massabuau, 1999, 2000). The final protocol will be termed LSD OT for Low Salt and Diet, Optimal Temperature.

2. Materials and methods

2.1. Facilities and material

The maturation tanks were circular in shape and made in Scobalite® (fibreglass sheet 1 mm thick). Two sizes of tank were used: 4-metre diameter tanks for females and 3-metre diameter tanks for males. All the tanks were 1.25 m high and held 50 cm of water. The bottom of the tanks was covered by a lower layer of gravel separated by Bidim® (geotextile) from an upper layer of sand. The total thickness of the substrate was 8–10 cm. Each tank was supplied with sea water through a PVC tube (4 cm diameter) with a shutter. A PVC tube drain (9 cm diameter) fixed vertically in the centre of each tank determined the water's height. Water renewal was continuous, at a rate of 50% per day for females and 100% for males. Each tank was also equipped with an air-lift system connected to a circular network of PVC tubes buried in the lower sediment layer. With this system, the water circulated from top to bottom through the sand base which thus acts as a mechanical filter. Additional aeration was