Abstract

Marine fish larvae are particularly vulnerable during their early stages of ontogeny due to strict larviculture requirements. Euryhaline rotifers *Brachionus*, especially those belonging to the *B. plicatilis* species complex are often used as the preferred initial-feed for most marine finfish species. Given current problems associated with differential growth and survival rates during hatchery culture, it is apparent that further research is required to investigate the use of enrichment media, the type of live feeds and the response by larvae during early larval ontogenetic development. This study used the L-type *B. plicatilis* and SS-type *B. rotundiformis* as the model rotifer species and the red sea bream *Pagrus major* and the Japanese flounder *Paralichthys olivaceus* as the model marine finfish species. The effects of different enrichment diets on population growth, fatty acid content and protein content of L and SS morphotypes of *B. plicatilis* sp. complex were investigated. Four common Japanese commercial rotifer enrichment formulations were tested: docosahexaenoic acid (DHA) enriched *Chlorella vulgaris*, frozen *Nannochloropsis oculata*, salmon roe emulsion oil, against a control diet of normal *C. vulgaris*. In both morphotypes, DHA-enriched *C. vulgaris* and frozen *N. oculata* had significantly higher population growth and fatty acid content compared to the negative impacts on population growth under salmon roe oil treatment. This is despite it having good levels of highly unsaturated fatty acids (HUFA). Variations in content of non-polar lipids and polar lipids between L and SS-type were observed. Differences were observed in soluble protein levels with higher levels detected in DHA-enriched *Chlorella* and control treatment. Using the two enrichment diets that had high levels of HUFA, DHA-enriched *C. vulgaris* and salmon roe oil, larviculture trials were conducted on *P. major* and *P. olivaceus* from 0-15 days after hatching (DAH) using L-type *B. plicatilis*. HUFA content was better in the combined salmon roe emulsion and DHA-enriched *C. vulgaris* treatment due to eicosapentanoic acid fortification from the roe emulsion. Despite this, the DHA-enriched *C. vulgaris* only treatment was found to

have higher population growth, egg bearing capacity, soluble protein and free amino acid content. Both *P. major* and *P. olivaceus* suffered high mortality under the combined diet. In contrast, better survival, generally higher gut content, high ingested soluble protein and trypsin activity fraction was observed in the DHA-enriched *C. vulgaris* only treatment. This could reflect its nutritional qualities as its high free amino acid and soluble protein composition are beneficial for early stage larvae due to ease of hydrolysis and absorption by the early digestive system. Using both L-type *B. plicatilis* and SS-type *B. rotundiformis* fortified on DHA-enriched *C. vulgaris*, larviculture trials were also conducted on *P. olivaceus* from 0-7 DAH to assess if there were any differences in protein hydrolysis and digestive trypsin activity at first feeding. At 5 DAH, hydrolysis was significantly higher in larvae fed SS-type whereas L-type treatment had not completely hydrolyzed the proteins after 3 hours at the same molecular weight (50 kDa). Larvae fed SS-type had significantly higher trypsin activity at 3-7 DAH. Contribution of live prey to trypsin fraction in larvae showed significantly higher fraction for SS-type at 5 DAH and 6 DAH. Exogenous trypsin contribution from live prey was relatively low when compared to the total trypsin activity in larvae. This study revealed that depending on dosage, morphotypes within *B. plicatilis* species complex do not respond well to oil-based enrichments despite their high HUFA content. This was also mirrored during larviculture of *P. major* and *P. olivaceus*. Furthermore, this study highlighted the adaptability of *P. olivaceus* to different rotifer sizes during its early developmental stages.