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Development of intensive copepod culture technology for *Parvocalanus crassirostris*: Optimizing adult density

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ABSTRACT

The use of copepod nauplii as live prey for first-feeding marine fish larvae is enabling the culture of many marine fish species with small, difficult to rear larvae. The small planktonic nauplii of the copepod *Parvocalanus crassirostris* is a particularly suitable first-feed due to its small size and ready acceptance by larvae of many species. This study details the relationship between stocking density and egg and nauplii production rates for *P. crassirostris* copepods, demonstrating a significant decline in culture production as system densities are increased. Fecundity decreased from 26 eggs female^{-1} day^{-1} at an adult density of 0.25 mL^{-1} to less than 1 egg female^{-1} day^{-1} when operated at 8 adults mL^{-1}. Effects of increasing adult densities on nauplii survival, feed availability, and water quality were sequentially investigated as potential mechanisms for the apparent inverse relationship between adult density and fecundity, with little success. In contrast, increasing egg and nauplii harvest frequency yielded large improvements in egg and nauplii production, with small (1 L) scale cultures yielding over 40,000 eggs and nauplii per day when stocked at 4 adults mL^{-1}. A 1500 L pilot production system, designed to efficiently remove eggs and nauplii, generated a mean daily output of 18 million eggs and nauplii per cubic meter of culture volume under continuous operating conditions for a period of over one month.

1. Introduction

Captive production, or aquaculture, of desirable marine species is increasingly viewed as a more sustainable alternative to harvesting wild stocks for both the seafood industry and aquarium trade. Indeed the number of marine species that can be cultured has increased dramatically over the past few decades. However, many marine species, particularly many of the reef-associated fishes, generate larvae that are too small to be cultured using rotifer- and *Artemia*-based hatchery technologies (Ostrowski and Laidley, 2001). After several decades of research, copepods, particularly the nauplii of calanoid copepods, are generally accepted to be of the appropriate size and nutritional value needed to support many of the challenging finfish species through the critical first-feeding period (Pepin and Penney, 1997; McKinnon et al., 2003; Drillet et al., 2006; Sampey et al., 2007). Examples of marine fishes that have now been successfully reared using copepod nauplii as first-feed include snappers (Doi et al., 1994; Payne et al., 2001; Ogle et al., 2005; Phelps and Sumiarsa, 2005), groupers (Doi et al., 1997; Toledo et al., 1999), seahorses (Payne and Rippingale, 2000a), killifish (Grageda et al., 2008), West Australian dhufish (Payne et al., 2001), Florida Pompano (Cassiano et al., 2011), marine angelfishes (Shields and Laidley, 2003; Laidley et al., 2004; Laidley et al., 2008; Baensch and Tamaru, 2009a,b; Leu et al., 2010), and the early stages of yellow tang (Laidley et al., 2011).

Investigators have used a range of approaches to obtain an adequate supply of copepods for larval rearing including the following: (1) sieving wild-plankton from natural waters (Quin, 1993; Van der Meeren and Naas, 1997; Baensch and Tamaru, 2009a,b), (2) propagation of mixed assemblages in outdoor tanks or ponds (Ogle, 1979; Ohno and Okamura, 1988; Su et al., 1997; Støttrup, 2000; Lemus et al., 2004; Ogle et al., 2005; Lindley and Phelps, 2009), and (3) intensive culture of single copepod species using intensive indoor culture systems (Sun and Fleeger, 1995; Schipp et al., 1999; Payne and Rippingale, 2001; Shields and Laidley, 2003; Shields et al., 2005; Puello-Cruz et al., 2009).

The collection of copepods from natural waters may be the easiest method, however, this approach requires hatchery operations to be located near appropriate marine ecosystems. Further, these natural sources exhibit large variations in both the composition and abundance of target live feed organisms (Bartholemew, 1973; Kimmerer, 1984). The use of extensive pond and other mesocosm systems provides more control over copepod availability, but the approach also requires ready access to coastal water sources for pond stocking and yields large fluctuations in species composition and production output (Lemus et al., 2004; Lindley and Phelps, 2009). Both wild-harvest and extensive pond systems are also prone to contamination by pathogens and other undesirable planktonic species (Phelps and Sumiarsa, 2005; Lahnsteiner et al., 2009). In contrast, intensive culture systems provide greater control over the composition, availability, and biosecurity of

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copepod supplies. Still, this technology is at an early stage of development and requires significant advancement prior to wide-spread commercial implementation (Schipp et al., 1999; Støttrup, 2000).

Optimization efforts for physical parameters such as salinity (Halsband-Lenk et al., 2002; Peck and Holste, 2006; Milione and Zeng, 2008; Ohs et al., 2010), temperature (Nagaraj, 1992; Chen et al., 2006; Holste and Peck, 2006; Milione and Zeng, 2008; Rhyne et al., 2009), photoperiod (Peck and Holste, 2006; Camus and Zeng, 2008; Peck et al., 2008), and biological parameters such as copepod nutrition (Berggreen et al., 1988; Kiørboe, 1989; Payne and Rippingale, 2000b; Hasset, 2004; Shields et al., 2005; Camus et al., 2009) have yielded improvements in culture productivity, however, intensive production of calanoid copepod nauplii to meet the feed requirements of a commercial hatchery is lacking. A major impediment to the wide-spread use of copepods in aquaculture remains the tendency for copepods to stop reproducing when cultured intensively (Støttrup and Norksker, 1997).

Reduced reproductive output at a high population density is a common ecological relationship for many terrestrial and aquatic species (Christian, 1971; Mueller, 1997; Murdoch, 1994; Krebs, 1995; Sibly and Hone, 2002). Density-dependent decreases in reproductive output have been documented in wild copepod populations (Sibly et al., 2000; Kiørboe, 2006) as well as laboratory monocultures including *Acartia tonsa* (Medina and Barata, 2004; Peck and Holste, 2006; Lemus, 2006; Jepsen et al., 2007), *Acartia sinjiensis* (Camus and Zeng, 2009), *Amphiascoides* sp. (Walker, 1979), *Bestiolina similis* (VanderLugt and Lenz, 2008), *Centropages typicus* (Miralto et al., 1996), *Tigriopus japonicus* (Kahan et al., 1988), and *Tisbe* spp. (Hoppenheit, 1976; Fava and Crotti, 1979; Zhang and Uhlig, 1993). Density effects observed are quite variable between species (and culture systems), however, the majority of these studies displayed reduced culture output at densities approaching 1 adult mL^{-1}. Although natural copepod populations are rarely thought to attain such high population densities overall (Houde, 1978), they are known to form swarms with densities well above 1 adult mL^{-1} both in the wild (Emery, 1968; Hamner and Carleton, 1979; Ueda et al., 1983) and in laboratory cultures (Buskey et al., 1996). The ability to maintain culture productivity at these higher densities (i.e. >1 adult mL^{-1}) would thus be a major advance toward cost-effective application of copepod-based live feeds technologies in the commercial culture of marine fishes.

Proposed mechanisms for this inverse relationship between adult density and fecundity include chemical cues (Katona, 1973; Fava and Crotti, 1979; Walker, 1979; Ban and Minoda, 1994; Lonsdale et al., 1998), water quality degradation (Ban and Minoda, 1994), maternal inhibition of egg development (Kahan et al., 1988), cannibalism (Williamson and Vanderploeg, 1988; Lazzaretto and Salvato, 1992; Daan et al., 1988; Bonnet et al., 2004; Basedow and Tande, 2006; Lemus, 2006; Camus and Zeng, 2009), physical disturbance (Peters and Downing, 1984; Miralto et al., 1996), and feed limitation (Kimmerer, 1984; Brand, 1985; Hopcroft and Roff, 1998; Guisande et al., 2000; Davis and Alatalo, 1992; Kiorboe and Nielson, 1994; Milione and Zeng, 2007). Although each of these mechanisms (dependent upon the species of copepod being cultured) can clearly affect culture productivity, none appears to fully explain the large drop-off in fecundity observed when cultures are stocked at desired (>1 mL^{-1}) culture densities.

Parvocalanus crassirostris is an herbivorous, paracalanid copepod found throughout the subtropics including Hawaiian waters (Bartholemew, 1973). Females are continuous broadcast spawners with eggs often produced in batches of four. Eggs are ca. 60 μm in diameter and take 6.75 h at 25 °C to hatch into non-feeding stage 1 nauplii (49 μm wide, 77 μm long; Kline, 2011). *P. crassirostris* begin feeding on phytoplankton as stage 3 nauplii (Lawson and Grice, 1973). Nauplii mature over 8–10 days (at 25 °C) into adults. Female *P. crassirostris* produce eggs until death (approximately 28 days post hatch) however greater fecundity is noticed with younger females. Adult male *P. crassirostris* have reduced mouth parts (Kline, 2011) and die within a week, presumably due to starvation. Adult *P. crassirostris* are sexually dimorphic with male bodies being both smaller in size and more ovoid in shape compared to females (Kline, 2011). *P. crassirostris* is a natural prey item of marine finfish larvae in the wild (Kimmerer and McKinnon, 1989; Allen et al., 1995; McKinnon et al., 2003) and has a nutritional profile suggested for larval fish as a feed (McKinnon et al., 2003). Larval fish successfully reared using *P. crassirostris* nauplii as a feed include the flame angelfish (*Centropyge loriculus*, Shields and Laidley, 2003), bluefin trevally (*Caranx melampygus*, Laidley et al., 2004), and red snapper (*Lutjanus campechanus*, Shields et al., 2005).

In this study the relationship between culture density and fecundity of locally isolated *P. crassirostris* was examined. The goals of this study were to (1) explore probable mechanisms (i.e., cannibalism, feed deficiency, chemical cues, and water quality) for observed decreases in *P. crassirostris* fecundity at the relatively higher adult stocking densities, (2) develop an optimized culture protocol based on these findings, and (3) design and test an integrated pilot-scale copepod production system capable of meeting the copepod eggs and nauplii requirements of a copepod-based marine finfish hatchery operation.

2. Materials and methods

2.1. Copepod culture

P. crassirostris were isolated from Kaneohe Bay (Oahu, HI) in 2004 and have since been maintained in small-scale master cultures. All cultures were maintained at 25 ± 1 °C in a temperature controlled room and fed daily with the diatom *Chaetoceros muelleri* and the haptophyte *Tisochrysis lutea* (formerly *Isochrysis galbana*) at densities of 150,000 cells mL^{-1} for each algae. Culture water was supplied from an onsite saltwater well and diluted to 22 ppt with municipal freshwater, mechanically filtered to 0.35 μm, and UV irradiated. Cultures received gentle aeration to facilitate mixing and gas exchange. Flasks and tanks received 24 h fluorescent lighting to facilitate in situ algae growth, with 1500 L "production" tanks receiving supplemental overhead illumination from 120 w compact fluorescent lights (ca. 5400 lx at water surface).

Master cultures were maintained in 2 L Erlenmeyer flasks that were harvested using a 38 μm nylon sieve every three to four days to exchange culture water and clean culture containers. Cultures were restocked with a mixture of copepod stages at a density of approximately 10 copepods mL^{-1}.

For general nauplii production, excess copepods from master cultures were scaled-up over time into larger 1500 L tanks. A two-stage production cycle was then initiated whereby harvested eggs and nauplii from 1500 L "production" tanks were stocked daily into individual 1000 L "maturation" tanks and matured to adulthood over 9 days. This cohort of recently matured adult copepods was then used in small-scale copepod trials or returned to production tanks to maintain adequate numbers due to attrition of older adult copepods over time. Cultures were harvested using nylon sieves to separately collect larger (>125 μm) adults and smaller (125 to 38 μm) eggs and nauplii.

2.2. Microalgae culture

Cultures of *C. muelleri* and *T. lutea* were obtained from the National Center for Marine Algae and Microbiota (CCMP strains 194 and 463 respectively) and gradually scaled up to 20 L polycarbonate carboys, followed by production in 290 L chemostats. Cultures were grown indoors under a 24L:0D photoperiod in water treated the same as described for *P. crassirostris* master and production cultures. Cultures were supplemented daily with F/2 media (Microalgae Grow Mass Packs, #F2A6, Pentair AES, Apopka, Florida, USA), however, the addition of silica did not appear to affect the growth of this strain of diatom and so was not added to cultures in order to reduce costs. Concentrations of