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Condition, survival and growth in situ of hatchery-reared stage IV lobster (*Homarus americanus*) fed *Artemia* and lipid-rich wild zooplankton

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ABSTRACT

The release of hatchery-reared American lobster (*Homarus americanus*) is used, on some occasions, as a tool to enhance depleted lobster stocks. Current Canadian lobster enhancement programs rely on the release of postlarvae (stages IV and V), which are still vulnerable to high mortality. We hypothesized that larvae enriched in triglycerides (TAG) and polyunsaturated fatty acids (PUFA) during their development from stage I to stage IV have higher survival and growth rates. We assessed the condition, growth and survival of lobster postlarvae (stage IV) fed during their larval stages with live natural zooplankton, to maximise TAG and PUFA enrichment, compared to those fed a standard *Artemia* diet. Stage I larvae obtained from ovigerous wild females were cultured in the hatchery on either an *Artemia* or a zooplankton diet until stage IV (7 d old), when they were transferred to the field (i) individually in small boxes for 3 months or (ii) communally in large enclosures for 3–4 months to assess survival and growth in situ. Our results show that a diet of natural zooplankton significantly increases the TAG and PUFA content of larvae compared to a diet of live *Artemia*. Stage IV lobsters fed natural zooplankton had a better condition index (TAG/ST) and a higher activity of the enzyme regulating osmoregulation than those fed *Artemia*. Larvae fed zooplankton had a significantly higher growth rate than larvae fed *Artemia*, (655 vs 507 μm dry mass d^{-1}, respectively). Despite these differences, survival was similar for both diets, in the hatchery as well as in situ, in predator-free situations. Nevertheless, a higher growth rate may be a potential survival advantage for newly released lobsters, allowing them to reach a size refuge from predation more rapidly. We conclude that even if live *Artemia* remains the best and most reliable source of live food for the production of American lobster larvae, it needs to be considerably enriched with PUFA, at levels comparable to that of natural zooplankton, to increase osmoregulation potential and growth.

1. Introduction

The release of hatchery-reared American lobsters (*Homarus americanus*) as a tool to increase depleted lobster stocks has been used in Atlantic Canada (Waddy and Aiken, 1998) and Maine, USA (Beal et al., 1998) in the early 1900s as well as in the 1970–1990s. More recently, following important declines in lobster landings in some areas of Atlantic Canada, there has been a renewed interest in releasing lobsters. However, there are still major constraints affecting the success of enhancement initiatives, and the development of such programs remains controversial (Araki and Schmid, 2010). In the few examples where field work was carried out to investigate the survival of released hatchery-reared American lobsters, recapture success was rather low (0.02%) (e.g. Castro et al., 2001). However, recent work by Comeau (2006) suggests more optimistic results, as one-year old lobsters in areas seeded the year before were seen in higher abundance than in control areas.

Canadian lobster enhancement programs rely mostly on the release of stage IV–V lobsters that are small and highly vulnerable to mortality. To increase the success of these programs, a best practice is to ensure that released lobsters are of high quality, i.e., that they have the potential to survive and grow in the wild. Here, we investigated how the quality of hatchery-produced lobster could be improved through a nutritional approach. In *H. americanus* larvae, triglycerides (TAG) constitute essential energetic reserves and have been shown to be a good proxy for the general physiological condition of larvae (Fraser, 1989; Harding and Fraser, 1999; Thériault and Pernet, 2007). TAG-enriched larvae are generally considered to have better chances of survival in stressful environments (Fraser, 1989; Liddy et al., 2004; Ouellet and Taggart, 1992; Ouellet et al., 1995). The fatty acid constituents of TAG and membrane phospholipids are also important for larval growth and survival, and several studies have focused on the importance of polyunsaturated fatty acids (PUFA) in larval nutrition (Glencross, 2009; Tocher et al., 2008). PUFA, such as docosahexanoic acid

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(22:6n-3, DHA), considered as essential for growth and survival, increase cellular membrane fluidity and associated enzyme activity (Else and Hulbert, 2003; Hall et al., 2002), facilitate metabolic activity (Else et al., 2004; Hulbert and Else, 1999), and therefore could favor the larval osmoregulatory processes (Charmentier et al., 2001) necessary to increase larval density at the moment of pelagic–benthic transition.

At present, a formulated feed qualitatively equivalent to live food is not available for lobster larvae (Fiore and Tlusty, 2005; Thériault and Pernet, 2007; Tlusty et al., 2005), and *Artemia* constitutes the main source of live food in lobster hatcheries (Fiore and Tlusty, 2005; Tlusty et al., 2005). In their recent work, Thériault and Pernet (2007) showed that lobster larvae fed with live *Artemia* were characterized by a dietary deficiency. To our knowledge, this is the only study characterizing the neutral (energetic lipids) and polar (membrane lipids) fraction of lipids in lobster larvae. They showed that the proportion of essential fatty acids (DHA, arachidonic acid (20:4n-6, AA) and eicosapentaenoic acid (20:5n-3, EPA)) in the polar lipids of lobsters was higher than in the neutral lipids and in the diets, suggesting selective incorporation into membrane phospholipids at the expense of reserve lipids. This selective retention response seems related to the too low dietary proportions of essential fatty acids in *Artemia*.

Preliminary observations made in 2007 showed that lobster larvae fed natural zooplankton had higher levels of TAG and neutral and polar PUFA, mainly DHA, than those fed live *Artemia* (R. Tremblay, Université du Québec à Rimouski, unpublished data). Moreover, zooplankton-fed larvae survived better during larval development. The objective of this current work was to evaluate TAG and PUFA enrichment in lobster larvae fed a regime based on natural zooplankton compared to a standard diet composed of a mixture of juvenile and adult *Artemia*. We hypothesized that larvae fed with natural zooplankton are richer in TAG and PUFA, mainly DHA, are characterized by higher activity of Na+/K+-ATPase (the main enzyme involved in osmoregulation process), and have higher survival and growth rates through the larval stages in the hatchery as well as in situ once released. Natural zooplankton is viewed here as a possible good alternative to enhance or perhaps replace an *Artemia* diet. In this study, the natural zooplankton diet was used also to validate if the absence of selective retention of essential fatty acids in the cell membrane is related to a better larval condition. Accordingly, this could help to optimize the fatty acid composition of a diet that would be more suitable for lobster larvae.

2. Material and methods

2.1. Larval culture

Lobster rearing was conducted in 2009 at the Centre aquacole marin de Grande-Rivière (CAMGR) of the Quebec Ministry of Agriculture, Fisheries and Food, in Grande-Rivière, Québec, Canada.

2.1.1. Broodstock

Ten berried females were caught by lobster fishermen off Grande-Rivière in mid-June during the 2009 lobster fishing season. The females were brought to CAMGR and quarantined in individual plastic pans in a tank filled with filtered seawater (10 μm) treated with ultraviolet light (UV). Water temperature was kept similar to ambient temperatures (12 °C). After one week, the females were transferred to three 130 L spawning tanks. Each tank contained three to four females separated by plastic walls. The flow-through system allowed water to be completely renewed every hour. The water drained from each tank was gravity-fed into a large 480 L retention tank, where newly hatched larvae from the different females were mixed, concentrated and then collected. The mean oxygen (±SD) concentration was maintained at 88.6 ± 5.2% and salinity (±SD) at 25.7 ± 1.8 ppt. The females were fed whole frozen shrimp and fish, and were exposed to a photoperiod of 16:8 h (light:dark). Water temperature was gradually increased to 20 °C (by 1 °C in the morning and 1 °C in the evening) to accelerate embryonic development and hatching.

2.1.2. Experimental design

The larvae were reared in ten 40 L planktonkreisels (1500 larvae per planktonkreisel) filled with filtered (10 μm), degassed, UV-treated seawater supplied at a rate of 1.5 L min^{-1} and maintained at 19.6 ± 0.1 °C. During the experiment, oxygen concentration was stable at 98.1 ± 2.1% and salinity at 28.7 ± 0.7 ppt. After a few days, the flow was increased to 2 L min^{-1} to reduce cannibalism. Three times a week, 10 larvae were taken out of the tanks to visually assess their development stage. When a postlarva (PL) (stage IV) was present in a tank, it was taken out and placed in an individual compartment 5.8 × 5.8 × 5.8 cm with a screen bottom. The compartments were placed on 2 cm diameter pipes in raceway tanks (to facilitate water circulation from the bottom). The raceway tanks contained 0.15 m^3 of seawater flowing at a rate of 1.65 L min^{-1}. Temperature was maintained at 19.7 ± 0.1 °C, oxygen at 90 ± 1.2%, and salinity at 28.4 ± 0.5 ppt. PL in compartments were fed individually twice a day (at 10:00 and 17:00 h), and water flow was momentarily stopped to facilitate food intake.

Five planktonkreisels were used for each diet (*Artemia* and natural zooplankton, see Sections 2.1.2.1 and 2.1.2.2). For each diet, food was placed in two 208 L tanks. Food was distributed daily in each planktonkreisel between 10:00 and 17:00 h using peristaltic pumps (MasterFlex Console Drive, Model 7520-40, Cole-Palmer Company, Vernon Hills, IL, USA). During food distribution and for the following 2 h, the drain of each planktonkreisel was covered with a NITEX mesh (100 μm) to minimize food loss. The flow of the food solution pumped from the 208 L tanks into the planktonkreisels was adjusted to maintain equivalent diets in terms of dry mass. The dry mass of each type of food was determined daily: 3 × 110 mL samples of zooplankton and 3 × 15 mL samples of *Artemia* were taken from the tanks, filtered on pre-weighed GF/C filters (Whatman International Ltd, Maidstone, UK), rinsed with a 3% ammonium formate solution, and dried at 70 °C for 16 h. Smaller volumes of *Artemia* were used because of their higher concentration. Three days a week, samples of zooplankton and *Artemia* were filtered on pre-combusted (at 450 °C) GF/C filters to determine the lipid content of each diet. Filters were stored in 4 mL amber glass vials with Teflon-lined caps under nitrogen in 2 mL of chloroform: methanol (2:1 v/v) at −80 °C until analysis.

Thirty 7 d old stage IV PL (PL_{7d}) were removed from the raceway tanks, gently rinsed with distilled water and dried on a clean paper towel. Ten were used to estimate dry mass after 48 h drying at 70 °C, ten were stored in 4 mL amber glass vials with Teflon-lined caps under nitrogen in 2 mL of chloroform:methanol (2:1 v/v) at −80 °C for lipid composition, and the remaining ten were stored at −80 °C immediately after sampling for determination of Na^+/K^+-ATPase enzymatic activity.

Survival between stages I and IV was estimated three times a week by visually counting all larvae in three 1 L subsamples collected in each experimental planktonkriesel. To estimate larval growth, ten larvae were randomly sampled in each experimental planktonkriesel and measured using a binocular (Wild Heerbrug) fitted with a digital camera (Cool Snap-Pro Color, Media Cybernetics) and image analysis software (Image Pro-Plus, Media Cybernetics). After 7 days, 7 d old stage IV PL (PL_{7d}) were placed either in boxes or enclosures for in situ experiments (see Section 3).

2.1.2.1. Artemia diet (ART). The first treatment consisted of a daily ration per larva of 80 freshly hatched (24–48 h) *Artemia* and 8 adult *Artemia* that had been enriched with Easy Selco (Inve Aquaculture Nutrition, Salt Lake City, UT, USA). *Artemia* cysts (Premium Great Salt Lake Artemia Cyst, Artemia International, Texas, USA) were hydrated and hatched in a culture cone and then transferred to 18 L carboys (freshly hatched *Artemia*) or 140 L kawal tubes for enrichment. *Artemia* adults were